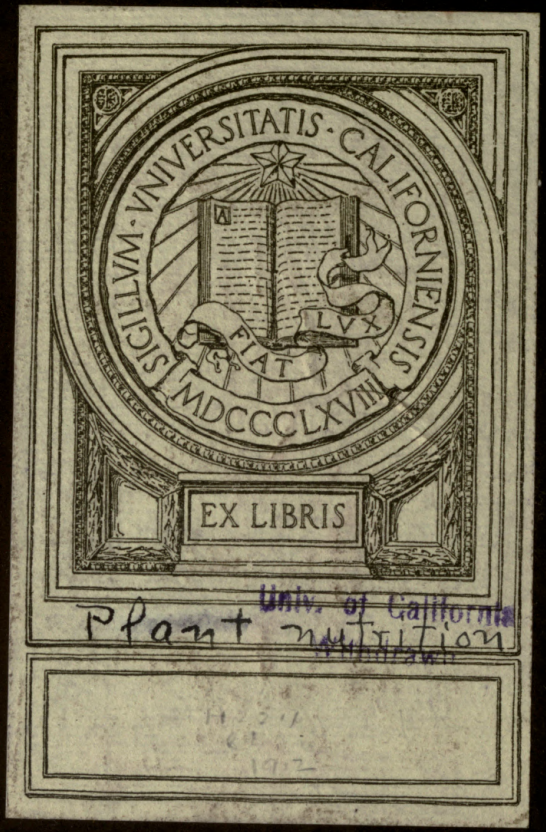

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A TEXT-BOOK

OF

PHYSIOLOGICAL CHEMISTRY

BY

OLOF HAMMARSTEN

EMERITUS PROFESSOR OF MEDICAL AND PHYSIOLOGICAL CHEMISTRY IN THE UNIVERSITY OF UPSALA

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Authorized Translation

FROM THE AUTHOR'S ENLARGED AND REVISED SEVENTH GERMAN EDITION

BY

JOHN A. MANDEL, Sc.D.

PROFESSOR OF CHEMISTRY IN THE NEW YORK UNIVERSITY AND BELLEVUE HOSPITAL MEDICAL COLLEGE

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PREFACE TO THE SEVENTH GERMAN EDITION.

This edition differs from the earlier ones by containing a new chapter on "Physical Chemistry in Biology." This chapter has been written by Professor S. G. Hedin, of Upsala. In order to give space for this new, second chapter, without changing the number of chapters of the earlier editions, the fifth chapter of these earlier editions on "the animal cell" has been dropped and its contents incorporated in the other chapters. For example, in this edition the nucleic acids, as well as the purine and pyrimidine bases, are treated of in connection with the nucleoproteins, and the phosphatides are discussed with the fats in Chapter V, a change which also seems necessary for other reasons. In general, the plan of the book remains unchanged. The numerous publications in physiological chemistry which have appeared since the publication of the sixth edition have necessitated a thorough revision and rewriting of all the chapters. Unfortunately a considerable increase in the size of the book could not be prevented.

Olof Hammarsten.

Upsala, November 16, 1909.

TRANSLATOR'S PREFACE TO THE SIXTH AMERICAN EDITION.

ALTHOUGH a number of excellent works on Physiological Chemistry have appeared during the past few years, HAMMARSTEN'S "Physiologischen Chemie" maintains its early popularity; and I am confident that all workers in biochemical research are thankful to Professor HAMMARSTEN for the labor and care that he has exercised in the prepartion of the present edition.

The work of translating and editing has been a labor of love, inasmuch as I feel that it will be of aid in the advance of this department of chemical science.

I am indebted to my assistant, Mr. A. O. GETTLER, for help given in revising the proof.

JOHN A. MANDEL.

NEW YORK, April, 1911

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PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

INTRODUCTION.

It follows from the law of the conservation of matter and of energy that living beings, plants and animals, can produce neither new matter nor new energy. They are only called upon to appropriate and assimilate material already existing and to transform it into new forms of energy.

Out of a few relatively simple combinations, especially carbon dioxide and water, together with ammonium compounds or nitrates, and a few mineral substances, which serve as its food, the plant builds up the extremely complicated constituents of its organism—proteins, carbohydrates, fats, resins, organic acids, etc. The chemical work which is performed in the plant must, therefore, in the majority of cases, consist in syntheses; but besides these, processes of reduction take place to a great extent. The radiant energy of the sunlight induces the green parts of the plant to split off oxygen from the carbon dioxide and water and this reduction is generally considered as the starting-point in the syntheses that follow. According to a hypothesis suggested by A. Baeyer,[1] formaldehyde is first produced, $CO_2 + H_2O = CH_2O + O_2$, which by condensation is transformed into sugar. From the sugar other bodies can then be built up.

With the aid of the silent electric discharge W. Loeb[2] has succeeded in obtaining from carbon dioxide and water, formaldehyde, and as a product of polymerization, also glycolaldehyde, $CH_2OH.CHO$, from which sugar can be readily produced. Still the conditions under which these bodies were formed cannot be applied to the conditions in the plants. The investigations of Usher and Priestley[3] are of greater interest

[1] Ber. d. d. chem. Gesellsch., 3.
[2] Zeitschr. f. Electrochem., 12.
[3] Proc. Roy. Soc. London, 78, Series B.

in that they show the formation of formaldehyde in the photolytic decomposition of moist carbonic acid in the presence of chlorophyll. These investigations also do not seem to be entirely free from exception. The conception as to the formation of sugar from formaldehyde is also often different from that explained by v. BAEYER's idea, and his view as to the assimilation of carbonic acid constitutes a hypothesis which requires further proof. The essentials of this hypothesis, namely, a formation of formaldehyde with a subsequent sugar formation by condensation of the aldehyde groups, is still very generally accepted as probably correct. Independent of the ways and means of how the assimilation processes in the plants originate, it is obvious that the free, radiant energy of the sun is hereby bound and stored in a new form, as chemical energy, in the combinations formed by the syntheses.

In animal life the conditions are not the same. Animals are dependent either directly, as the herbivora, or indirectly, as the carnivora, upon plant-life, from which they drive the three chief groups of organic nutritive matter—proteins, carbohydrates, and fats. These bodies, of which the protein substances and fats form the chief mass of the animal body, undergo within the animal organism a cleavage and oxidation, and yield as final products exactly the above-mentioned chief components in the nutrition of plants, namely, carbon dioxide, water, and ammonia derivatives, which are rich in oxygen and have little energy. The chemical energy, which is partly represented by the free oxygen and partly stored up in the above-mentioned more complex chemical compounds, is transformed into other forms of energy, principally heat and mechanical work. While in the plant we find chiefly reduction processes and syntheses, which by the introduction of energy from without produce complex compounds having a greater content of energy, we find in the animal body the reverse of this, namely, cleavage and oxidation processes, which, as we used to state, convert chemical tension into living force.

This difference between animals and plants must not be overrated, nor must we consider that there exists a sharp boundary line between the two. This is not the case. There are not only lower plants, free from chlorophyll, which in regard to chemical processes represent intermediate steps between higher plants and animals, but the difference existing between the higher plants and animals is more of a quantitative than of a qualitative kind. Plants require oxygen as peremptorily as do animals. Like the animal, the plant also, in the dark and by means of those parts which are free from chlorophyll, takes up oxygen and eliminates carbon dioxide, while in the light the oxidation processes going on in the green parts are overshadowed or hidden beneath the more intense reduction processes. As in the animal, we also find a heat production in fermentation produced by plant organisms; and even in a few of the higher

plants—as the *aroideæ* when bearing fruit—a considerable development of heat has been observed. On the other hand, in the animal organism, besides oxidation and splitting, reduction processes and syntheses also take place. The contrast which seemingly exists between animals and plants consists merely in that in the animal organism the processes of oxidation and splitting are predominant, while in the plant chiefly those of reduction and synthesis have thus far been studied.

WÖHLER[1] in 1824 was the first to observe an example of the SYNTHETICAL PROCESSES within the animal organism. He showed that when benzoic acid is introduced into the stomach, it reappears as hippuric acid in the urine after combining with glycocoll (aminoacetic acid). Since the discovery of this synthesis, which may be expressed by the following equation:

$$\underset{\text{Benzoic acid}}{C_6H_5.COOH} + \underset{\text{Glycocoll}}{NH_2.CH_2.COOH} = \underset{\text{Hippuric acid}}{NH(C_6H_5.CO).CH_2.COOH} + H_2O,$$

and which is ordinarily considered as a type of an entire series of syntheses occurring in the body, where water is eliminated, the number of known syntheses in the animal kingdom has increased considerably. Many of these syntheses have also been artificially produced outside of the organism, and numerous examples of animal syntheses of which the course is absolutely clear will be found in the following pages. Besides these well-studied syntheses, there also occur in the animal body similar processes unquestionably of the greatest importance to animal life, but of which we know nothing with positiveness. We enumerate as examples of this kind of synthesis the re-formation of the red-blood pigment (the hæmoglobin), the formation of the different proteins from simpler substances, and the production of fat from carbohydrates. This last-mentioned process, the formation of fat from carbohydrates, is also an example of reduction processes which occur to a considerable extent in the animal body.

Formerly the view was generally accepted that ANIMAL OXIDATION takes place in the fluids, while to-day we are of the opinion, derived from the investigations of PFLÜGER and his pupils,[2] that it is connected with the form-elements and the tissues. The question as to how this oxidation in the form-elements is induced and how it proceeds cannot be answered with certainty.

When a substance is oxidized by neutral oxygen at the ordinary temperature or at the temperature of the body, the substance is said

[1] Berzelius, Lehrb. d. Chemie, übersetzt von Wöhler, **4**, p. 356, Abt. 1, Dresden, 1831.

[2] Pflüger, Pflüger's Archiv, **6** and **10**; Finkler, *ibid.*, **10** and **14**; Oertmann, *ibid.*, **14** and **15**; Hoppe-Seyler, *ibid.*, **7**.

to be easily oxidized or autoöxidized, and the process is considered as a direct oxidation or autoöxidation. As the oxygen of the inspired air, and that of the blood, is neutral molecular oxygen, the old assumption that ozone occurs in the organism has now been discarded for several reasons. On the other hand, the chief groups of organic nutritives, carbohydrates, fats, and proteins, the last two forming the chief mass of the animal body, are not autoöxidizable substances. They are on the contrary bradoxidizable (TRAUBE) or dysoxidizable bodies. They are nearly indifferent to neutral oxygen, and it is therefore a question how an oxidation of these and other dysoxidizable bodies is possible in the animal body.

In explanation it is very generally admitted that the oxygen is made active and this causes a secondary oxidation. It is generally conceded that in autoöxidation a cleavage of neutral oxygen takes place. The autoöxidizable substance splits the oxygen molecule and combines with one of the oxygen atoms, while the other free atom as active oxygen may oxidize the dysoxidizable substances simultaneously present. Such a subordinate oxidation is called an indirect or secondary oxidation. The explanation of animal oxidations has been attempted in different ways by the supposition that the oxygen is made active and thus produces secondary oxidation.

The cause of the animal oxidation is considered, by PFLÜGER and several other investigators, to be dependent upon the special constitution of the protoplasmic proteins or the living protoplasmic substance. This investigator calls the proteins outside of the organism, or those which occur in the animal fluids, "non-living proteins," and considers them to be somewhat different from those occurring in living protoplasm. The latter are called "living proteins" (PFLÜGER), "active proteins" (LOEW), or "biogens" (VERWORN). The living protoplasmic molecule differs from the ordinary non-living protein by being more unstable and therefore having a greater inclination toward intramolecular changes of the atoms.

The reason for these greater intramolecular movements PFLÜGER ascribes to the presence of cyanogen, and LATHAM attributes it to the presence of a chain of cyanalcohols in the protein molecule. VERWORN,[1] on the contrary, claims an intramolecular introduction of oxygen into a large hypothetical protoplasmic molecule, the "biogen molecule," which is supposed to contain a nitrogen or an iron complex as an oxygen receptor or carrier, and a side-chain of aldehydic character like that of the carbohydrates, as an oxidizable group.

According to LOEW,[2] who bases his claim upon special investigations and

[1] Pflüger, Pflüger's Archiv, **10**; Latham, Brit. Med. Journal, 1886; Verworn, Die Biogenhypothese, Jena, 1903.

[2] Loew and Bokorny, Pflüger's Archiv, **25**; O. Loew, *ibid.*, **30**; and specially O. Loew, Die chemische Energie der lebenden Zellen. 2. Aufl. Stuttgart, 1906.

numerous toxicological observations, the unstability of the active protein molecule is due to the simultaneous presence of aldehyde and unstable amino groups. These occur separated from each other in the active proteins, and when they combine the protoplasm dies, the molecule being changed into a stable condition, i.e., into dead protein. It is also a fact that all substances which react with aldehyde and unstable amino groups are poisonous to the living cells.

Loew has also shown, in conjunction with Bokorny, that in many plants a very unstable reserve-protein substance occurs, which to a certain extent occupies an intermediate position between protein and organized living substance.

The explanation as to the oxidation process differs entirely according to the conception of the structure of the unstable protoplasmic molecule. If the living protoplasmic protein is not, like protein in the ordinary sense, indifferent to neutral oxygen, we can admit of a cleavage of the oxygen molecule by this change. The protein would be itself oxidized, while on the other hand a secondary oxidation of other difficultly oxidizable substances could be brought about by the oxygen atoms set free.

Another very widely diffused view exists in regard to the origin of the activity of the oxygen, namely, that by the decomposition processes in the tissues, reducing substances are formed which split the neutral oxygen molecule, uniting with one oxygen atom and setting the other free.

The formation of reducing substances during fermentation and putrefaction is generally known. The butyric fermentation of dextrose in which hydrogen is set free—$C_6H_{12}O_6 = C_4H_8O_2 + 2CO_2 + 2H_2$—is an example of this kind. Another example is the appearance of nitrates in consequence of an oxidation of nitrogen in cases of putrefaction, which process is ordinarily explained by the statement that reducing, easily oxidizable bodies are formed which split oxygen molecules, liberating oxygen atoms which afterward oxidize the nitrogen. It is assumed also that the cells of the animal tissues and organs have the power, like these lower organisms which produce fermentation and putrefaction, of causing splitting processes in which easily oxidizable substances, perhaps also nascent hydrogen (Hoppe-Seyler[1]), are produced.

In accordance with what has been stated above on the oxidations in the animal body, primarily a cleavage of the organic constituents of the body takes place with the formation of readily oxidizable substances. The oxidation of these latter produces an activation of the oxygen and hence may also cause a secondary oxidation of dysoxidizable substances. The products formed by these splittings and oxidations may perhaps in part be burned within the body without undergoing further cleavage, but more probably they must first undergo a further cleavage

[1] Pflüger's Archiv, **12**.

and then succumb to consecutive oxidations, until after repeated cleavages and oxidations the final products of metabolism are formed.

An activation of the oxygen may be produced according to O. NASSE[1] by a hydroxylization of the constituents of the protoplasm with the splitting off of molecules of water. If benzaldehyde is shaken with water and air, an oxidation of the benzaldehyde into benzoic acid takes place, while oxidizable substances present at the same time may also be oxidized. The simultaneous presence of potassium iodide and starch or tincture of guaiacum causes a blue coloration because the hydroxyl (OH) takes the place of the hydrogen in the aldehyde group, and these two hydrogen atoms, one derived from the aldehyde and the other from the water, have a splitting action on the molecular oxygen. NASSE and RÖSING [2] have also found that certain varieties of protein have the property of being hydroxylized in the presence of water. According to NASSE a whole series of oxidations in the animal body may be accounted for by the oxygen atoms set free in hydroxylization similar to that of benzaldehyde. In opposition to this view we must remark that the oxidation of benzaldehyde to benzoic acid may also take place in other ways, thus by the intermediary formation of a peroxide (see BAEYER and VILLIGER; ENGLER and WEISSBERG [3]).

By quantitative methods, VAN'T HOFF and his pupils [4] have shown that molecular oxygen can be divided in two parts by certain autoxidation processes. One of these parts unites with the autoöxidizer and the other with a body simultaneously present but not directly oxidizable, which, according to the suggestion of ENGLER,[5] is called the acceptor. VAN'T HOFF claims that the oxygen molecule dissociates at ordinary temperatures into minimum quantities of positively and negatively charged oxygen atoms, the ions of similar charge uniting with the autoöxidizable substance, while the remaining ions oxidize the acceptor. Such a division of the oxygen into halves has also been shown by other investigators, such as MANCHOT, ENGLER, and his collaborators.[6] These investigators nevertheless consider that autoöxidation takes place in another way, namely, by the formation first of peroxides by the taking up of oxgyen molecules.

TRAUBE [7] has also expressed a similar view. According to him, in autoöxidation we have to deal, in the first place, not with a cleavage of the oxygen, but with a splitting of water in which the hydroxyl groups of the water combine with the oxidizable substance, while the hydrogen

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[1] O. Nasse, Rostocker Zeitung, No. 534, 1891, and No. 363, 1895.

[2] E. Rösing, Untersuchungen über die Oxydation von Eiweiss in Gegenwart von Schwefel. Inaug. Dissert. Rostock, 1891.

[3] Baeyer and Villiger, Ber. d. d. chem. Gesellsch., **33**; Engler and Weissberg, *ibid.*, **33**.

[4] van't Hoff, Zeitschr. f. physikal. Chem., **16**; Jorissen, Ber. d. d. chem. Gesellsch., **30**, and Zeitschr. f. physikal. Chem., **22**; Ewan, *ibid.*, **16**.

[5] Ber. d. d. chem. Gesellsch., **33**.

[6] Manchot, Ueber freiwillige Oxydation. Leipzig, 1900; Engler and Weissberg, Ber. d. d. chem. Gesellsch., **33**; Engler and Frankenstein, *ibid.*, **34**.

[7] Ber. d. d. chem. Gesellsch., **15**, **18**, **19**, **22**, and **26**.

atoms set free on the decomposition of the water unite with the neutral oxygen, forming hydrogen peroxide, which may naturally also have an oxidizing action.

$$A+2H_2O+O_2=A(OH)_2+H_2O_2.$$

According to the generally accepted view, which is based upon the work of BACH, MANCHOT, ENGLER and collaborators,[1] the oxygen acts also in the autoöxidation in the first place as an entirely inactive molecule. According to ENGLER and his collaborators, at least in the simplest cases ("direct autoöxidation" according to ENGLER), the oxygen molecules unite with the activating body (A), forming a peroxide-like substance which can give up one of the two oxygen atoms to an acceptor (B):

$$A+O_2=AO_2 \text{ and } AO_2+B=AO+BO.$$

The appearance of a peroxide has, at least in certain cases, been shown, as, for instance, with dimethylfulvene C_8H_{10} where the peroxide $C_8H_{10}O_4$ occurs (Engler). The autoöxidation may also in other cases proceed otherwise, as shown by Manchot[2] in the oxidation of hydro-compounds like oxanthranol, which proceeds according to the equation $AH_2+O_2=A+H_2O_2$, namely, with the formation of a hydroperoxide. This is different from TRAUBE'S views.

If this is so, still we do not know to what extent such peroxides are formed in oxidation in the living cell. The possibility of a production of peroxides, and also of hydrogen peroxide, in animal oxidation is still generally admitted, and CHODAT and BACH[3] have indeed been able to show a peroxide formation in plants. Still, if hydrogen peroxide were formed in such oxidations it would have no further physiological importance, according to LOEW, because the animal and plant cells contain special enzymes, called by him *catalases*, which quickly decompose the hydrogen peroxide with the production of molecular oxgyen. According to LOEW[4] the physiological importance of the catalases is to protect the cell from hydrogen peroxide, which acts as a protoplasmic poison, a view which is accepted by many investigators but still disputed by others.

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[1] Engler and Wild, Ber. d. d. chem. Gesellsch., **30**; Bach, Le Moniteur scientifique, 1897, and Compt. rend., **124**; Manchot, l. c.

[2] Verhandl. d. Phys. med. Gesellsch. zu Würzburg (N. F.), Bd. **39**.

[3] Ber. d. d. chem. Gesell., **35** u. **36**.

[4] Loew, U. S. Dept. of Agriculture, Rep. **68**, 1901, and Ber. d. d. chem. Gesellsch., **35**; in regard to the opposed views see Chodat and Bach., l. c., Bach, Monit. scientif. (4) **20**; Kastle and Loevenhart, Amer. Chem. Jour., **29**; Herlitzka, Chem. Centralbl., 1908; Euler, Hofmeister's Beiträge, **7**, and especially O. Loew, Centralbl. f. Bacteriol., **21**; Abt. **2**, which contains the literature.

LOEW,[1] who has also opposed the view as to the oxygen becoming active with the setting free of oxygen atoms, has sought for the reason of the oxidations in the unstable properties of the protoplasmic proteins. The active movement of the atoms within the active protein molecule is transmitted to the oxygen and to the oxidizable substance, and when this dissolution of the molecule has proceeded to a certain point the oxidation occurs by virtue of the chemical affinity. The reason for this unstable condition of living protein molecules has already been given above.

SCHMIEDEBERG,[2] who also denies the supposition that the oxygen becomes active, is of the view that the tissues by the mediation of the oxidations do not increase the oxidizing activity of the oxygen, but more probably act on the oxidizing substances, making them more susceptible to oxidation.

All the views presented thus far assume a continuous oxidation of the primary active substance. The view has also been suggested that animal oxidation may be brought about by oxygen-carriers, i.e., by bodies which, according to the older views, without being oxidized themselves, act in a manner analogous to that of the nitric oxide in the manufacture of sulphuric acid by alternately taking up and giving off oxygen in the oxidation of dysoxidizable bodies. TRAUBE many years ago explained the oxidations of the animal body in this way, and he called these questionable oxygen-carriers *oxidation ferments*.[3]

It is a fact that in the animal and plant kingdom bodies have been extensively found which can bring about oxidations and which in many respects behave like ferments or enzymes. It is therefore necessary, before we proceed further, that we discuss the peculiar and highly important bodies that have been called ferments or enzymes. The nature, properties and mode of action of these bodies will be discussed somewhat in detail in a following chapter (II), but in order to understand what follows, we will give a short summary of the subject.

Alcoholic fermentation by yeast and other processes of fermentation and putrefaction are dependent upon the presence of living organisms, ferment fungi, and splitting fungi of different kinds. The ordinary view, according to the researches of PASTEUR, is that these processes are to be considered as phases of the life of these organisms. The name *organized ferments* or *ferments* has been given to such micro-organisms, of which ordinary yeast is an example. However, the same name has also been given to certain bodies or mixtures of bodies of unknown organic origin

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[1] Die chem. Energie d. lebenden Zellen. 2. Aufl. Stuttgart, 1906.

[2] Arch. f. exp. Path. u. Pharm., **14**.

[3] M. Traube, Theorie der Fermentwirkungen. Berlin, 1858.

which are products of the chemical work within the cell, and which after they are removed from the cell still have their characteristic action. Such bodies—for example, malt diastase, rennin, and the digestive ferments—are capable in the very smallest quantity of causing a decomposition or cleavage in very considerable quantities of other substances, without entering into permanent chemical combination with the decomposed body or with any of the cleavage or decomposition products. These formless or *unorganized ferments* are generally called *enzymes*, according to KÜHNE.

A ferment in a more restricted sense is therefore a living being, while an enzyme is a product of chemical processes in the cell, a product which has an individuality even without the cell, and which may be active when separated from the cell. The splitting of invert-sugar into carbon dioxide and alcohol by fermentation is a fermentative process closely connected with the life of the yeast. The inversion of cane-sugar is, on the contrary, an enzymotic process caused by one of the bodies or a mixture of bodies formed by the living ferment, which can be separated from this ferment, and still remain active even after the death of the latter. Consequently ferments and enzymes are capable of manifesting a different behavior toward certain chemical reagents. Thus there exist a number of substances, among which we may mention arsenious acid, phenol, toluene, salicylic acid, boracic acid, sodium fluoride, chloroform, ether, and protoplasmic poisons, which in certain concentration kill ferments, but which do not noticeably impair the action of the enzymes.

The above view as to the difference between ferments and enzymes has lately been essentially shaken by the researches of E. BUCHNER[1] and his pupils. He has been able to obtain from beer-yeast, by grinding and strong pressure, a cell-fluid rich in protein, and which when introduced into a solution of a fermentable sugar caused a violent fermentation. The objections raised from several sides that the fluid expressed still contained dissolved living cell substance has been so successfully answered by BUCHNER and his collaborators that there is at present no question that alcoholic fermentation is caused by a special enzyme or mixture of enzymes called *zymase*, which is formed in the yeast-cell.

As from the yeast cells so also from other lower organisms, indeed

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[1] E. Buchner, Ber. d. deutsch. chem. Gesellsch., **30** and **31**; E. Buchner and Rapp, *ibid.*, **31**, **32**, **34**; H. Buchner, Stizungsber. d. Gesellsch. f. Morphol. u. Physiol. in München, **13**, 1897, part 1, which also contains the discussion on this topic. See also E. and H. Buchner and M. Hahn, Die Zymasegärung, München, 1903; Stavenhagen, Ber. d. deutsch. chem. Gesellsch., **30**; Albert and Buchner, *ibid.*, **33**; Buchner, *ibid.*, **33**; Albert, *ibid.*, **33**; Albert, Buchner, and Rapp, *ibid.*, **35**; in regard to the opposed views see Macfadyen, Morris, and Rowland, *ibid.*, **33**; Wroblewski, Centralbl. f. Physiol., **13**, and Journ. f. prakt. Chem. (N. F.), **64**.

from the lactic-acid bacilli and beer vinegar bacteria, it is possible to separate the specific fermentative principle of these organisms from the living organism and to bring about changes with the dead organism (E. BUCHNER, and MEISENHEIMER and GAUNT, HERZOG [1]). The question whether there exist ferment processes which, in PASTEUR'S sense, are the result of the biological phenomena connected with the metabolism of the micro-organism and which we can directly identify with the life processes, is very difficult to answer; hence for the present we have no foundation for a sharp differentiation between the organized ferments and enzymes. The metabolic processes of the living organisms which we recognize as fermentation phenomena must as a rule be ascribed to enzymes acting within the cell. If such processes are closely connected with the life of the cell, then this is explained in part by the fact that this special enzyme is produced only by living cells and in part by the fact that it cannot be separated from the living cells or that it is readily destroyed on the death of the cell.

All enzymes and ferments, both names having now the same significance, are considered as organic substances formed in the cells but whose chemical nature has not been determined for the present.

We have no characteristic reaction for all enzymes or ferments in general, but each enzyme is characterized by its specific action and by the conditions under which it operates. Of special importance is, first, the fact that the enzymes do not form permanent chemical combinations in definite proportions by weight with the bodies upon which they act, or their decomposition products; and, secondly, that an insignificantly small amount of the enzyme can decompose a relatively enormous amount of substance. For instance, 1 part of invertin can invert 100,000 parts of cane-sugar (O'SULLIVAN and THOMPSON [2]), and 1 part of chymosin can in a short time decompose more than 400,000 parts of casein (HAMMARSTEN [3]). This does not exclude the possibility of a primary, but temporary, combination of the enzymes with the substances acted upon—a process which is highly probable from the numerous observations which will be discussed in Chapter II.

The specific action of the enzymes is of special importance, as one and the same enzyme acts only upon one substance or a definite group of substances. Their action seems to be entirely dependent upon the stereometric construction of the substance acted upon, which will be discussed in Chapter II.

The relationship of the enzymes to the inorganic catalysts is also of

[1] E. Buchner and J. Meisenheimer, Ber. d. d. chem. Gesellsch., **36**; and Annal. d. Chem. u. Pharm., **349**; with Gaunt, *ibid.*, **349**; Herzog, Zeitschr. f. physiol. Chem., **37**.

[2] O'Sullivan and Thompson, Journ. of Chem. Soc., **57**.

[3] See Maly's Jahresbericht, **7**.

the greatest importance. The catalysts, like the enzymes, are not found in the final products of the reaction, they are not used up in the process, and the quantity of the active substance proportionate to the quantity of substance transformed is infinitesimally small in enzyme action as well as in catalysis. Without having positive proof, nevertheless we now consider that enzyme action is not to be considered as the starting of a reaction which would not of itself take place, but rather as an acceleration of a slowly proceeding, often not noticeable, chemical change. According to this conception enzyme action comes in line with catalysis, as understood to-day (see Chapter II).

An enzyme is an organic substance formed in an animal or plant cell, which is destroyed by heating its aqueous solution and which acts like the catalytes, but only upon certain bodies. Some restriction must be put to this, as the cells do not always produce a complete enzyme, but more often only the mother-substance thereof. These mother-substances of the enzymes are called *proenzymes* or *zymogens*. The zymogens are under certain conditions converted into enzymes, and in certain cases this is brought about by the special action of bodies called *kinases*, which have been little studied (see Chapter II, VI and IX).

According to their action most of the enzymes which have been studied may be divided into two chief groups, namely, those having a *hydrolytic* action and those having an *oxidizing* action.

After this short discussion of the enzymes we can now return to the oxidations and the so-called oxidation ferments.

It has also been positively proven by the researches of Jaquet, Salkowski, Spitzer, Röhmann, Abelous and Biarnès, Bertrand, Bourquelot, De Rey-Pailhade, Medwedew, Pohl, Jacoby, Chodat and Bach [1] and others that in the blood and different tissues of the animal body, as also in plant-cells, substances occur which have the property of causing certain oxidations. For this reason these bodies have been called *oxidases*, and they have been divided into two different groups. The ferments of the first group, called primary or direct oxidases, or simply *oxidases*, transfer the oxygen of the air directly to other bodies. Those of the second group, the indirect oxidases or *peroxidases*, are active only in the presence of a peroxide, as they set oxygen free

[1] Jaquet, Arch. f. exp. Path. u. Pharm., **29**; Salkowski, Centralbl. f. d. med. Wissensch., 1892 and 1894, and Virchow's Arch., **147**; Spitzer, Pflüger's Archiv, **60** and **67**; Spitzer and Röhmann, Ber. d. deutsch. chem. Gesellsch., **28**; Abelous et Biarnès, Arch. de physiol. (5), **7**, 8, and **9**, and Compt. rend. soc. biol., **46**; Bertrand, Arch. de physiol. (5), 8, **9**, and Compt. rend., **122**, **123**, **124**; Bourquelot, Compt. rend. soc. biol., **48**, and Compt. rend., **123**; Medwedew, Pflüger's Arch., **81**; Jacoby, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, which contains the literature of the subject; Chodat and Bach, l. c.

from these latter by decomposition. Correspondingly the oxidases turn tincture of guaiac blue directly, while the peroxidases only have this action in the presence of a peroxide. The *catalases* do not give any reaction with guaiac either directly or indirectly in the presence of peroxides.

According to the investigations of BACH and CHODAT[1] the conditions are otherwise. According to the observations they have made upon plants, there exist no oxidases, and what has been described under this name is only a mixture of oxygenases and peroxidases. The *oxygenases* are of a protein nature, contain manganese or iron, and are converted into peroxides after taking up oxygen. These peroxides themselves have only a slight oxidizing power, but are made active by the peroxidases. The peroxidases, which do not have the slightest oxidizing power in the absence of peroxides, are not proteins. In oxidation, according to the hypothesis of BACH and CHODAT, the molecular oxygen is first converted by the oxygenase into peroxide. This peroxide is activated by the peroxidase and then has strong oxidizing power. The oxidizing power of the so-called direct oxidases is brought about by a combined action of the oxygenases and peroxidases.

The chemical nature of the oxidation enzymes is still unknown, and the statements on this subject are very contradictory. Certain oxidases are proteins, namely, nucleoproteins (SPITZER), others globulins (ABELOUS and BIARNÈS), and still others, like the liver aldehydase (JACOBY) and laccase (BERTRAND), are of a non-protein nature. The materials upon which the oxidation enzymes act may also be very different from each other. Thus the oxidases studied by RÖHMANN and SPITZER may by synthetical oxidation produce indophenol from α-naphthol and p-phenylenediamine in the presence of alkali. The salicylase or aldehydase detected in the liver and many other organs oxidizes many aldehydes to their corresponding acids, but does not give the indophenol reaction. The laccase isolated by BERTRAND from the juice of the lac-tree has an oxidizing action upon polyhydric p-phenols, such as hydroquinone, but not upon tyrosine. The bodies called tyrosinases, first found by BERTRAND[2] in certain fungi and later also found by BIEDERMANN, v. FÜRTH, and SCHNEIDER[3] in the animal kingdom, have, on the contrary, an action upon tyrosine, converting it into colored compounds. Another oxidase occurring in the liver and spleen, and called xanthine-oxidase by BURIAN, has the property, as shown by SPITZER,

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[1] Biochem. Centralbl., **1**, pp. 417 and 457.

[2] In regard to the work of the various authors cited, see footnote 1, p. 11.

[3] Biedermann, Pflüger's Archiv, **72**; v. Fürth and Schneider, Hofmeister's Beiträge, **1**.

WIENER, SCHITTENHELM, and BURIAN,[1] of transforming xanthine and hypoxanthine into uric acid by oxidation.

Like other enzymes in general the so-called oxidation enzymes show from the above a more or less pronounced specificity, as a certain oxidation enzyme acts only upon certain substances and does not oxidize others. This behavior, which is difficult of reconciliation, that these enzymes act as oxygen-carriers, indicate that in the oxidation the active substances do not act upon the oxygen, but rather upon the substance to be oxidized. We cannot at present give any statement as to the extent of action of the oxidation enzymes in the oxidations of the animal body, and it is still a question whether we were actually dealing with enzymes in all cases where oxidation enzymes have been claimed to have been found.

In investigations with hydroperoxides and vegetable peroxidases BACH and CHODAT[2] found that peroxides and peroxidases always took part in the reaction in constant proportions, and that the peroxidases were quickly used up, which certainly does not indicate that these bodies have an enzymotic nature. ASO[3] has also shown that in certain cases where an apparent oxidase action was present, we were very probably dealing only with nitrites which were present; and finally, attention must be called to the fact that manganese or iron, sometimes in considerable amounts, has been found in many oxidases. As manganous and ferrous salts are active as catalytes in certain other oxidations, so also in certain cases important rôles as oxygen-carriers have been ascribed to these metals, for instance in laccase, which contains manganese (BERTRAND), and the oxidases containing iron (SPITZER'S nucleoprotein). MANCHOT[4] by his work on the autoöxidation of ferrous sulphate has called attention to the apparently great importance of iron for physiological oxidations, and recently several investigators as TRILLAT, DONY-HÉNAULT and J. WOLFF, have shown that several oxidase actions can be brought about by colloidal inorganic catalytes or by mixtures of these with organic colloids. Finally EULER and BOLIN[5] have shown that the lucerne-laccase is not an enzyme at all, but that its action is due to the presence of salts of organic acids. The question as to the nature of the so-called oxidation enzymes surely requires a thorough investigation.

The assumption of special-oxidation enzymes does not at least give

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[1] Spitzer, Pflüger's Archiv, **56**; Wiener, Arch. f. exp. Path. u. Pharm., **42**; Schittenhelm, Zeitschr. f. physiol. Chem., **42** and **43**; Burian, *ibid.*, **43**.

[2] Ber. d. d. chem. Gesellsch., **37**.

[3] Beihefte zum botan. Centralbl., **18**.

[4] Zeitschr. f. anorg. Chem., **27**.

[5] Trillat, Compt. rend., **137** and **138**; Dony-Hénault, Bull. Acad. Roy. Belg. 1908; J. Wolff, Compt. rend., **146**; H. Euler and J. Bolin, Zeitschr. f. physiol. Chem., **57**.

us a sufficient explanation of the oxidative processes in the animal body, and the various divergent views as to the nature of these show us strikingly how little is positively known about these processes. There is no doubt that the animal body possesses in the so-called oxidation enzymes important means of bringing about oxidative decomposition of various substances, and the occurrence of numerous intermediary metabolic products in the animal body teaches us that the oxidation of the constituents of the body is not instantaneous and sudden, but takes place step by step, and hand in hand with cleavages. For a long time the oxidations in the animal body have been considered as a combustion, and such a conception is easily reconcilable with the above-mentioned views. In combustion in the ordinary sense, as, for example, the burning of wood or oil, we must not forget that the substances themselves do not combine with oxgyen. It is only after the action of heat has decomposed these bodies to a certain degree that the oxidation of the products of such decomposition takes place and is accompanied by the phenomenon of light. The above-mentioned specificity of the oxidation enzymes and also the recent observation that indeed rather simple cleavage products are burnt with difficulty in the organism and also that of two optical antipodes of an amino-acid, for example of leucine, the one (*l*.-leucine) is readily burnt while the other (*d*-leucine) is burnt with difficulty and only incompletely, seem to make it very probable that in many cases a very intense cleavage is necessary before oxidation occurs.

Most investigators are agreed that these decompositions are similar to certain oxidations studied by DREchsel [1] outside the animal body, where oxidations and reductions alternate in quick succession. The views are divided in regard to the manner and origin of this cooperative action.[2]

As the oxidations are explained by the action of special enzymes, so also special reducing enzymes, so-called *reductases* or *hydrogenases*, have also been accepted. Certain investigators claim that the so-called *philothions*, which develop hydrogen sulphide in the presence of sulphur and water, belong to this group, while others, on the contrary, do not accept this view, but consider the enzymotic nature of the philothions as doubtful.[3] The investigations of NASSE and RÖSING [4] on the oxi-

[1] Journ. f. prakt. Chem. (N. F.), **22**, **29**, **38**, and Festschrift für C. Ludwig, 1887.

[2] See M. Nencki, Arch. des sciences biol. de St. Pétersbourg, **1**, 483; Abelous and Aloy, Compt. rend., **136**, **137**; Kastle and Elvove, Amer. Chem. Journ., **31**; Underhill and Closson, Amer. Journ. of Physiol., **13**.

[3] De Rey-Pailhade, Recherches expér. sur le Philothion, etc., Paris (G. Masson), 1891, and Nouvelles recherches sur le Philothion, Paris (G. Masson), 1892; Bull. soc. chim. (4) I; Pozzi-Escot, Bull. soc. chim. (3), **27**, and Chem. Centralbl., 1904, **1**, p. 1645; Chodat and Bach, Ber. d. d. chem. Gesellsch., **36**; Abelous and Ribaut, Compt. rend., **137**, and Bull. soc. chim., Paris (3), **31**.

[4] See footnote 2, p. 6.

dation of protein in the presence of sulphur, speak against the enzymotic nature of this formation of hydrogen sulphide and the recent investigations of HEFFTER[1] have shown that certain reductions occurring in the tissues cannot be produced by enzymes. He has shown that those reductions which are not influenced by HCN, such as the reduction of pigments (methylene blue) can be brought about by the unstable H of sulphhydric compounds. For example, cysteine (see Chapter III), which quickly reacts with sulphur with the formation of H_2S, acts in this manner, and he has also shown the presence of similarly acting substances in various organs and organ extracts. Here we have a group of reductions which are not enzymotic. The reduction of nitrates seems at least in part, according to the investigations of VOGELSOHN,[2] not to be brought about by enzymotic substances.

There is no doubt that reductions occur to a great extent in the animal body and often hand in hand with oxidations; nevertheless the question as to how far special reduction enzymes take part in these reductions is still an open one. According to ABELOUS and ALOY[3] we have indeed enzymes that have an oxidizing as well as a reducing action, for they obtain the oxygen necessary for the oxidation of one body by removing it from another substance through reduction. Still this question requires further study.

The essential source of heat and mechanical work developed in the organism is to be found in the oxidations. Chemical energy is transformed into the above-mentioned forms of energy in cleavage processes, where complicated chemical compounds are reduced to simpler ones, and therefore the atoms change from an unstable to a stabler equilibrium, and stronger chemical affinities are satisfied. The animal body may also have a source of energy in the cleavage processes which are not dependent on the presence of free oxygen. The processes taking place in the active muscle are examples of this kind. A removed muscle, which gives off no oxygen when in a vacuum, may, as HERMANN[4] has shown, work, at least for a time, in an atmosphere devoid of oxygen, and give off carbon dioxide at the same time.

The cleavage processes are not only of especially great importance for the digestion of the foodstuffs and for their availability for the animal body, but also are important for the metabolic processes. If

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[1] Mediz. naturw. Arch., **1**, p. 81–104, Marburg; cited in Chem. Centralbl., 1907, p. 822.

[2] Ueber die Einwirkung von Organextrakten auf Nitrate und Nitrite, Inaug.-Dissert., Bern, 1907.

[3] Compt. rend., **136**, **137** and **138**. See also Abelous and Gerard, *ibid.*, **129**, on the reductases; Pozzi-Escot, Bull. soc. chim. (3), **27**.

[4] Untersuch. über den Stoffwechsel der Menschen, Berlin, 1867.

a cleavage process is connected with a decomposition of water and a taking up of its constituents it is called a hydrolytic cleavage. As an example of such a cleavage we can mention the decomposition of starch into sugar and the splitting of neutral fats into the corresponding fatty acids and glycerin:

$$\underset{\text{Tristearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{Glycerin}}{C_3H_5(OH)_3} + 3\underset{\text{Stearic acid}}{(C_{18}H_{36}O_2)}.$$

As a rule the hydrolytic cleavage processes as they occur in the animal body may be performed outside of it by means of higher temperatures with or without the simultaneous action of acids or alkalies. Considering the two above-mentioned examples, we know that starch is converted into sugar when it is boiled with dilute acids, and also that the fats are split into fatty acids and glycerin on heating them with caustic alkalies or by the action of superheated steam. The heat or the chemical reagents which are used for the performance of these reactions would cause immediate death if applied to the living body. Consequently the animal organism must have other means at its disposal which act similarly, but in such a manner that they may work without endangering the life or normal constitution of the tissues. The animal body has in the enzymes such means and especially the second chief group which have a hydrolytic action.

Among the hydrolytic enzymes we must mention in the first place the *proteolytic*, or those which dissolve protein, whose best studied representatives, pepsin and trypsin, occur in the animal kingdom; the *lipolytic* or fat-splitting; and the *amylolytic* enzymes, or *diastases* which act upon the complex carbohydrates. In this group we include the *amylases*, which act upon starch, and the *invertases*, which split the disaccharides into simpler forms of sugar. In close relation to those enzymes we may mention the *glucoside-splitting* enzymes, which occur especially in the higher plants. Among the hydrolytic enzymes of the animal kingdom we must also include *arginase*, which splits arginine into urea and ornithine; the two deamidizing enzymes *adenase* and *guanase*, which convert the two bodies adenine and guanine, with the splitting off of ammonia, into hypoxanthine and xanthine respectively; and the hippuric-acid-splitting *histozyme* and the urea-splitting *urease*. The *protein-coagulating* enzymes, *chymosin* or casein-coagulating, and *thrombin* or blood-coagulating enzyme, belong to a special though not clearly defined group. These enzymes are also included by some investigators among the proteolytic enzymes.

Many enzymes are secreted by the cells as such or as proenzymes. They act outside of the cells in which they were formed, or they act after

having been transformed into the enzyme, and hence are called secretion enzymes or *extracellular* enzymes.

Besides these extracellular enzymes we also have another group which act within the cells, hence are intracellular and therefore are called *intracellular* enzymes or *endoenzymes*. Numerous enzymes besides the yeast zymase belong to this group, and seemingly also oxidases and enzymes having hydrolytic action. The best studied of this group are the proteolytic enzymes, which were first observed by SALKOWSKI and his pupils, and which bring about the self-digestion or autodigestion of organs in the absence of micro-organisms. This *autodigestion* has been the subject of numerous investigations, principally by the HOFMEISTER school and especially by JACOBY.[1] The latter has given the name *autolysis* to the process, and he has shown that the enzymes taking part in this action do not come from the digestive tract and are not pepsin or trypsin taken up by the cells. In autolysis we are not only dealing with a proteolysis, but several other processes occur, such as the splitting of fats and carbohydrates, oxidations and reductions, and perhaps also syntheses.

We therefore generally designate as autolysis all the enzyme actions which take place in removed organs or fluids without the aid of micro-organisms, but it must not be forgotten that autolytic processes may also occur *intra vitam* under certain conditions. The combined action of various enzymes in autolysis also explains to us why, as especially shown by LEVENE and by JONES,[2] the products obtained by the hydrolytic cleavage of an organ by means of an acid are somewhat different from those products produced on autolysis.

It is at present impossible to state what part autolytic processes take in life under physiological conditions, and we can have only conjectures on this subject. In the autolysis of a removed organ or of one through which the blood is not flowing, the conditions in many ways are quite different from the conditions in life. The products which appear after weeks or months of autolysis, sometimes in very small quantities, do not give any clue to the nature of the processes, and conclusions must be drawn very carefully from these results.

The post-mortem autolyses, as far as studied, are chiefly proteolyses, but we must not forget that the enzymes taking part are in many cases most active in acid reaction, while they have only a weak action or

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[1] A complete summary of the literature of intracellular enzymes and autolysis may be found in Jacoby, Ueber die Bedeutung der intrazellulären Fermente, etc., Ergebnisse der Physiologie, Jahrg. **1**, Abt. 1, 1902. See also Preti, Zeitschr. f. physiol. Chem., **52**; Arinkin, *ibid.*, **53**; Hedin, Hammarsten's Festschr., 1906.

[2] Levene, Amer. Journ. of Physiol., **11** and **12**, and Zeitschr. f. physiol. Chem., **41**; W. Jones, *ibid.*, **42**.

are inactive in neutral or alkaline reaction. The observations of LANE-CLAYPTON and SCHRYVER,[1] that the autolysis of the liver and kidney begins only after a latent period of from two to four hours subsequent to the removal of the organ, are also of interest. The investigations of WIENER [2] show that the post-mortem formation of acid is the important factor in this, and according to HEDIN [3] it is due to the removal of the retarding action. HEDIN has shown by experiments with various organs that a preliminary treatment with acetic acid markedly helps the autolysis in alkaline reaction and he has also shown, especially for the enzyme of the spleen (*d*-lienase) that an anti-body of the enzyme is hereby made inactive.

The action of an enzyme can be retarded or arrested by another body which has been called an *antienzyme*, but the action of these has not been closely studied. They are perhaps of the greatest importance in life in retarding or regulating the action of the intra-cellular enzymes. Experience has shown that the post-mortem autolytic process may also be influenced by many other bodies and indeed in various ways. For example, according to HESS and SAXL, arsenious acid exerts a retarding action on the first stages of autolysis, while phosphorus accelerates it. Autolysis may also be influenced by toxins (see Chapter II) (HESS and SAXL [4]) and indeed so that first a retardation of the cleavage of the proteins takes place and then a strong acceleration.

It is difficult to judge of the importance of the autolytically active proteolytic enzymes for the physiological life of the cells, but there does not seem to be any doubt as to the importance of these enzymes in pathological conditions.

The changes of the liver and blood in acute phosphorus intoxication and in acute yellow atrophy of the liver, where we find in the urine the enzymotic decomposition products of the proteins, are examples of an *intra vitam* autolysis which is considered by some as an abnormal rise in the physiological autolysis. Another example is the solution of pneumonic infiltrations by the enzymes of the migrated and inclosed leucocytes as studied by FR. MÜLLER,[5] and this is at the same time an example of *heterolysis*, i.e., of a solution or a destruction in an organ by enzymes not belonging therein but introduced from without. An autolysis, although not very marked, occurs in those organs or parts of organs which have not been normally nourished because of a disturbance in

[1] Journ. of Physiol., **31**.

[2] Centralbl. f. Physiol., **19**, p. 349.

[3] Hammarsten's Festschr., 1906.

[4] Zeitschr. f. exp. Path. u. Therap., **5**, and Wien. klin. Wochenschr., **21**.

[5] Verhandl. d. naturforsch. Gesellsch. zu Basel, 1901. See also O. Simon, Deutsch Arch. f. klin. Med., 1901.

the circulation, and they are gradually consumed by this action. The part injured undergoes solution, while the healthy part remains unattacked. By this solvent action as well as by the formation of bactericidal bodies, as observed by CONRADI,[1] and of antitoxins (BLUM[2]) by means of autolysis, we can consider this autolysis as a remedy and perhaps also as a protective agent for the animal body.

For the present it is impossible to judge of the importance of the enzymes active in autolysis for physiological conditions, but this does not exclude the possibility that in normal cell life the enzymes play a very important rôle. Numerous observations show this to be true, and we tend more and more toward the view that the chemical transformations in the living cells are brought about by enzymes, and that these latter are to be considered as the chemical tools of the cells (HOFMEISTER and others[3]).

From this standpoint the enzymes are of especial interest because to-day it is the general belief that nearly all chemical processes of great importance do not occur in the animal fluids, but on the contrary in the cells, which are the real chemical workshops of the organism. It is also chiefly the cells, which by their more or less active efficiency regulate the extent of the chemical processes and thereby also the intensity of the general metabolism.

The researches into the chemical composition of the cells must therefore be of the greatest importance, but the difficulties which such investigations entail are very striking. The chemical investigations of animal cells must in most cases be connected with the study of those tissues whose chief part they represent. Only in a few cases, such for example as the investigation of pus or tissues very rich in cells, can the cells be directly or by relatively simple manipulation, be isolated in a rather pure form from the other parts of the tissues. Even in these cases the chemical investigation does not give any positive results as to the constituents of the living uninjured cells. If the physiological conditions of life of the cells are removed, or injurious external influences such as the action of high temperatures or of chemical agents are applied, then the protoplasm dies. The protoplasm, which in the generative cell during life forms a semi-solid mass which is contractile under certain conditions and which readily changes, consists, with the exception of the water, chiefly of protein substances, namely of colloids. On the death of the cell these proteins coagulate, at least in part, and other chemical transformations also occur in the cell. The alkaline reaction of the cells to

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[1] Hofmeister's Beiträge, **1**.

[2] *Ibid.*, 5, p. 142.

[3] F. Hofmeister, Die chemische Organisation der Zelle, Braunschweig, 1901.

litmus may change to an acid reaction due to the formation of paralactic acid. Also on the death of the cell other new bodies may be produced, or cell constituents, like glycogen, may be consumed and also constituents may pass from the cell into the surrounding fluid and hence be lost for the investigation.

There are still other difficulties that must not be underestimated. The constituents occurring in the cells are not all of the same physiological importance. Some of these are essentially necessary for the life of the cells, while others are only considered as stored-up reserve material or as metabolic products. In this connection we have only been able, thus far, to learn of certain substances which seem to occur in every developing cell. Such bodies, called PRIMARY by KOSSEL,[1] are, besides water and certain mineral constituents, proteins, nucleoproteins or nucleoalbumins, phosphatides (lecithin), glycogen (?), and cholesterin. Those bodies which do not occur in every developing cell are called SECONDARY. Among these we have fat, glycogen (?), pigments, etc. It must not be forgotten that it is still possible that other primary cell constituents may exist, as yet unknown to us, and we also do not know whether all the primary constituents of the cell are necessary or essential for its life and functions.

Another important question is the division of the various cell constituents between the two morphological components of the cell, namely, the protoplasm and the nucleus. This is very difficult to decide, for many of the constituents and even the division of the chief chemical constituent, the proteins, between the protoplasm and nucleus, have been but little studied. It is very probable that the nucleus (in abundance) as well as the protoplasm contains the conjugated proteins, which have been called nucleoproteins, and which will be discussed in detail in a following chapter (III). The nucleoproteins of the protoplasm are richer in the protein component and poorer in the phosphorized component as compared with the nucleoproteins of the nucleus, which also have a more marked acid character.

The question as to the occurrence of a special external boundary layer in the cells is of importance for the understanding of the metabolism of the cells, as well as for our knowledge as to the manner in which the intake and output of bodies transpires in the cells. In this connection we must call attention to the fact that in certain cells an external thick layer or a true membrane exists which seems to consist of protein substance. Even cells in which no special external boundary layer can be observed are still considered as having such a layer because of the permeability conditions.

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[1] Verhandl. d. physiol. Gesellsch. zu Berlin, 1890-91, Nos. 5 and 6.

NERNST[1] has shown by a special experiment that the permeability of a membrane for a certain substance is essentially dependent upon the solvent power of the membrane for the said substance. This point, which is of the greatest importance in the study of osmotic phenomena in living cells, has been specially investigated by OVERTON.[2] The behavior of the living cells toward dyestuffs, also the ready introduction into animal and plant protoplasm of such bodies as are insoluble or only slightly soluble in water but readily soluble in fats or fat-like bodies, has led OVERTON to conclude that the protoplasm-boundary layer behaves like a substance layer whose solvent power is closely related to the fatty oils. According to this investigator, the protoplasm-boundary layer is probably impregnated with *lipoids*, i.e., bodies which in regard to solubility and solvent power are more or less similar to the fats. The lipoids are not a class of bodies that can be defined chemically. The nature of certain of them is still not well known; but the best-known bodies, such as lecithin (the phosphatides especially) and cholesterin, must be considered as of the greatest importance.

The occurrence of an accumulation of lipoids as a special external boundary layer in the cells is not a sufficiently proven fact, and at least does not apply to all animal cells, and is not necessarily important in the explanation of the action of lipoids. Objections have also been made by many investigators to OVERTON'S theory, which has received rather general acceptation.[3] It does not apply to all cases; for example, according to COHNHEIM, it does not explain the absorption in the intestinal canal, and according to MOORE and ROAF it does not explain certain properties of the cells, namely, the different composition of the electrolytes within and without the cells and the selective taking up of certain soluble substances, such as foodstuffs, therepeutic agents, toxines, and antitoxines, on the part of the cells. The researches of these last investigators are based essentially upon the behavior of mineral bodies, and they show that the above theory presents certain difficulties in the understanding of the very important exchange of mineral bodies between the cells and the external fluid.

This leads us to the question as to the importance of water and the mineral bodies, which are of just as great moment for the life of the cells and their metabolism as the organic constituents. In regard to the water this follows from the fact that the animal body consists of

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[1] Zeitschr. f. physikal. Chem., **6**.

[2] Vierteljahrsschr. d. Naturf. Ges. in Zurich, **44** (1899), and Overton, Studien über die Narkose, Jena, 1901.

[3] See O. Cohnheim, Die Physiologie der Verdauung u. Ernährung, 1908. J. Loeb in Oppenheimer's Handbuch der Biochem., **2**, p. 105. T. B. Robertson, Journ. of Biol. Chem., **4**, 1908; B. Moore and H. Roaf, Biochem. Journ., **3**, 1908.

about two-thirds water. If we also recall that water is of the very greatest importance for the normal physical condition of the tissues, that the solution of numerous bodies and the dissociation of chemical compounds, that all flow of juices, all exchange of material, all supply of food, all growth or destruction and all removal of destructive products, are connected with the presence of water, and that besides this the water by its evaporation is an important regulator of temperature, it is evident that water must be a necessity of life.

The mineral substances found habitually in the cells of higher plants and of animals are *potassium, sodium, calcium, magnesium, iron, phosphoric acid, sulphuric acid, chlorine,* and perhaps also *iodine* (JUSTUS).[1] Besides, in certain cells or organs we also find *manganese, lithium, barium, silicium, fluorine, bromine,* and *arsenic.*

We are chiefly indebted to LIEBIG for showing that the mineral bodies are as important for the normal constitution of the organs and tissues, as well as for the normal performance of the processes of life, as the organic constituents of the body. The importance of the mineral constituents is evident from the fact that we know no animal tissue and no animal fluid which is free from mineral bodies, and also from the fact that certain tissues or tissue elements contain chiefly certain mineral bodies and not others. In regard to the alkali compounds this division is, in general, as follows: The sodium compounds occur chiefly in the fluids, while the potassium compounds occur especially in the form-elements. Corresponding to this, the cells contain chiefly potassium as phosphate, while they are less rich in sodium and chlorine compounds. Still we have some exceptions to this rule, and it must be remarked that BEEBE[2] has found considerably more sodium than potassium in malignant tumors.

The division of the mineral bodies in the various parts of a cell or a tissue is very difficult to determine, but in certain cases by means of micro-chemical reactions this can be determined in a way. For example, MACALLUM[3] has been able to show such a division in the case of potassium. According to him the potassium is absent in the cell nuclei and in the head of the spermatozoa as well as in the nerve cells and the axis-cylinders, while it occurs on the contrary in the medullary sheath and especially in the region of the nodes of RANVIER.

[1] Justus, Virchow's Arch., **170**, **176** and **190**. In regard to arsenic see the works of Gautier, Compt. rend., **129**, **130**, **131**, **139**; Bertrand, *ibid.*, **134**; Segale, Zeitschr. f. physiol. Chem., **42**; Kunkel, *ibid.*, **44**. In regard to the barium see Schulze and Thierfelder, Sitzungsber. d. Gesellsch. naturforsch. Freunde, 1905, No. 1, and in regard to lithium see Hermann, Pflüger's Arch., **109**; and in regard to manganese see Bradley, Journ. of Biol. Chem., **3**.

[2] Amer. Journ. of Physiol., **11** and **12**.

[3] Journ. of Physiol., **32**.

In the ordinary method of determining the mineral bodies in cells, fluids and tissues, namely by incineration, no positive knowledge can be acquired as to the division of the mineral bodies or their form of combination. On incineration we obtain not only a mixture of the mineral bodies of the nucleus and protoplasm, but, as is true for all animal fluids and tissues, the original relationship is markedly changed. The combinations between the colloidal and mineral substances are destroyed, carbon dioxide discharged, and sulphuric acid and phosphoric acid may be produced from the organic bodies. The ordinary chemical analysis is not sufficient for the study of the mineral constituents of the fluids or tissues, their forms of combination and action; hence we must resort to physical-chemical methods.

As a result of the investigations carried on by these methods, the conclusion has been reached, irrespective of the importance of the mineral bodies for the osmotic tension in the cells and tissues, that the part taken by the mineral bodies in cell life is essentially dependent upon the action of the ions. The investigations of Maillard on the toxic action of copper salts, and of Paul and Krönig [1] on that of mercury salts, acids, and alkalies, offer examples. From these investigations it follows that the toxicity is dependent upon the dissociation, and that it is not dependent upon the total amount of, for example, copper or mercury salts present in the solution, but rather upon the number of copper or mercury ions.

Important and conclusive investigations, which are considered as beautiful and instructive examples of the importance of ions for cell life, have been carried out by Loeb and his collaborators. Some of these examples will be discussed in the next chapter (II).

The chief mass of the cells consists of colloids, and as the normal function of the cells is connected with a certain physical condition of the protoplasm, it is natural to consider the action of ions in relationship to the changes in the condition of the colloids. These colloids can be precipitated by electrolytes, and it seems as if we are here dealing with ion action. A physiologically balanced salt mixture suitable for the normal functions may also be produced by the antagonism of the ion action in a complex solution containing several salts (Loeb). Changes in one or the other direction must correspondingly also bring about changes in the state of the colloid by the action of the ions. The action of ions in these cases, as well as the nature of colloids and the reasons for the change in their conditions, is a very difficult question, and this will be discussed in the following chapter (II).

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[1] Maillard, Journ. de Physiol. et Path., **1**; Paul and Krönig, Zeitschr. f. physikal. Chem., **12**, and Zeitschr. f. Hygiene, **25**.

As above stated, the chemical processes in animals and plants do not stand in opposition to each other; they offer differences indeed, but still they are of the same kind from a qualitative standpoint. PFLÜGER believes that there exists a blood-relation between all living cells of the animal and vegetable kingdoms, and that they originate from the same root. The animal body is a complex of cells, hence study of the chemical processes must not only be made upon higher plants, but also upon unicellular organisms in order that we get a proper explanation of the chemical processes in the animal organism. Although a biochemical study of the micro-organisms is very important, we must bear in mind also the important rôle played by such organisms in animal life, chiefly as exciters of disease; hence the study of the conditions of life of these micro-organisms and the chemical investigation of the products produced by them must be of infinite importance.

The products produced by micro-organisms may be of very different kinds. Among the substances produced in the decomposition of animal fluids and tissues by putrefactive organisms we find those having a basic nature. To this class belong the cadaver alkaloids called *ptomaines*, first found by SELMI in human cadavers and then specially studied by BRIEGER and GAUTIER.[1] Certain of these are poisonous, designated as *toxines*, while others are non-poisonous. They all belong to the aliphatic compounds and generally do not contain oxygen. As an example of these basic substances we must mention the two diamines, *cadaverine* or pentamethylenediamine, $C_5H_{14}N_2$, and *putrescine* or tetramethylenediamine, $C_4H_{12}N_2$, which have awakened special interest because they occur in the contents of the intestine and in the urine in certain pathological conditions, especially in cholera and cystinuria.[2] The putrefaction bases *marcitine*, $C_8H_{19}N_3$, *putrine*, $C_{11}H_{26}N_2O_3$, and *viridinine*, $C_8H_{12}N_2O_3$ isolated by ACKERMANN, also belong to this group. Of special interest is the bacterial poison isolated by FAUST[3], called *sepsine*, $C_5H_{14}N_2O_2$, which is the substance producing the characteristic toxic action of putrefactive masses. Sepsine was prepared by FAUST as a crystalline sulphate which, on repeated evaporation of its solution, was readily converted into cadaverine sulphate.

Those substances of basic nature which are incessantly and regularly

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[1] Selmi, Sulle ptomaine od alcaloidi cadaverici e loro importanza in tossicologia, Bologna, 1878; Ber. d. d. chem. Gesellsch., **11**, Correspond. by H. Schiff; Brieger, Ueber Ptomaine, Parts 1, 2, and 3, Berlin, 1885–1886; A. Gautier, Traité de chimie appliquée à la physiologie, **2**, 1873, and Compt. rend., **94**.

[2] See Brieger, Berlin. klin. Wochenschr., 1887; Baumann and Udransky, Zeitschr. f. physiol. Chem., **13** and **15**; Brieger and Stadthagen, Berlin. klin. Wochenschr., 1889.

[3] Faust, Arch. f. exp. Path. u. Pharm., **51**; Ackermann, Zeitschr. f. physiol. chem., **54** and **57**.

produced as products of the decomposition of the protein substances in the living organism, and which therefore are to be considered as products of the physiological metabolism, have been called *leucomaines* by GAUTIER[1] in contradistinction to the ptomaines and toxines produced by micro-organisms. These bodies, to which belong several well-known animal extractives, are considered by many as being of certain importance in causing disease. It has been contended that when these bodies accumulate on account of an incomplete excretion or oxidation in the system, an autointoxication may be produced (BOUCHARD and others).[2]

Of especially great interest are the toxines which are found in the higher plants and animals, like the jequirity-bean and castor-seed, in the poison of snakes and spiders, in blood-serum, etc., and particularly those produced by pathogenic micro-organisms have an unmistakable relation to the enzymes. A closer study of these various bodies, lysines, agglutinines, toxines, etc., as well as of the antitoxines and the theory of immunity, does not lie within the scope of this work, but on account of the great importance of the subject it will be briefly discussed in the next chapter (II).

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[1] Bull. soc. chim., **43**, and A. Gautier, Sur les alcaloïdes dérivés de la destruction bacterienne ou physiologique des tissues animaux, Paris, 1886.

[2] Bouchard, Leçons sur les auto-intoxications dans les maladies, Paris, 1887. See also the various text-books of clinical medicine.

CHAPTER II.

PHYSICAL CHEMISTRY IN BIOLOGY.

I. OSMOTIC PRESSURE.

When certain substances are placed in contact with water they dissolve therein and finally a liquid is obtained which contains an equal quantity of the dissolved substance in each unit volume. There exists between the water and the soluble body a certain attractive force. Upon this force depends also the so-called diffusion, which manifests itself when two different solutions of the same or different substances are brought into immediate contact with each other. The dissolved molecules and the water intermingle with each other so that finally the dissolved bodies are equally divided in the entire quantity of water. Imagine a cane-sugar solution in contact with pure water; the equilibrium or the homogeneity of the system can then be brought about in two ways; namely, the sugar molecule can migrate in part into the water, and secondly, the water can pass into the solution. If the two fluids at the beginning are in immediate contact with each other then the two processes take place simultaneously.

The conditions change when the two liquids are separated from each other by a membrane, which allows of the passage of water but not of the dissolved substance (in this case cane-sugar). In the presence of such a so-called semipermeable membrane the equilibrium can only be established by the water passing into the cane-sugar solution. Semipermeable membranes have been artificially prepared, and they also occur in nature, or conditions exist which give results like those of the membranes. To the first group belong Traube's so-called precipitation membranes.[1] For example, such a membrane can be produced by carefully dropping a concentrated solution of copper sulphate into a dilute solution of potassium ferrocyanide. Thereby the drop of copper sulphate is surrounded by a membrane of copper ferrocyanide, which is impervious to copper sulphate as well as to potassium ferrocyanide, but allows water to pass. The drops retain their blue color in the yellow solution but increase in volume, due to the taking up of water, until the tension of the membrane prevents the further increase in size. If the difference in

[1] Arch. f. (Anat. u.) Physiol., 1867, pages 87 and 129.

concentration of the two solutions is great enough, the membrane is ruptured by the pressure.

In order to give the copper-ferrocyanide membrane a greater rigidity, PFEFFER has suggested forming the precipitate on a porous, rigid wall.[1] For this purpose he makes use of a small, porous earthenware cell which, after careful cleaning, is treated with copper sulphate and potassium ferrocyanide so that the membrane is precipitated on the inner wall of the cell. The membrane thus obtained is impervious to the cane sugar. If the cell is filled with a cane-sugar solution and then placed in pure water, no sugar leaves the cell, while water passes into the cell, and this continues until the opposite pressure produced prevents the further passage of water. If the cell is completely closed and in connection with a manometer, then on the establishment of an equilibrium the manometer indicates the force with which the inclosed solution attracts water, or the *osmotic pressure* of the solution. For dilute cane-sugar solutions the osmotic pressure is approximately proportional to the concentration, which is shown by the following figures.

Conc. (c)	Osmotic Pressure (p)	$\frac{p}{c}$
1 per cent	53.5 cm. Hg.	53.5
2 "	101.6 "	50.8
4 "	208.2 "	52.1
6 "	307.5 "	51.3

The osmotic pressure rises slowly with the temperature.

Experiments with other semipermeable membranes have also been carried out by DE VRIES, and these will be discussed on page 30. DE VRIES' experiments have led to the following result: *Solutions of analogously constructed bodies having the same molecular concentration give the same osmotic pressure.*

VAN'T HOFF first called attention to the analogy which exists between the laws of osmotic pressure of a dissolved substance and of gases,[2] namely, that the osmotic pressure is proportional (or inversely proportional to the volume of the solution) to the concentration, and corresponds completely with BOYLE-MARIOTTE'S law on the relation between the volume and pressure of gases. Also, that equimolecular solutions have the same osmotic pressure, corresponds to AVOGADRO'S law, that equal volumes of different gases under the same pressure contain the same number of molecules. GAY-LUSSAC'S law that the pressure of a gas changes in proportion to the absolute temperature cannot be absolutely proven for the osmotic pressure because of the inaccuracy of the methods.

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[1] Osmotische Untersuchungen, Leipzig, 1877.

[2] Zeitschr. f. physik. Chem., 1, 481, 1887.

From PFEFFER'S results of the osmotic pressure of cane-sugar solutions VAN'T HOFF has calculated that it is the same as the pressure exerted by any gas of the same molecular concentration and temperature. In general the following is true:

Dissolved bodies exert in solution the same osmotic pressure they would exert if they were gases at the same temperature and in equal volume.

As the gas pressure, according to the kinetic theory of gases, is caused by the impact of the gas molecule upon the walls of the vessel, so also the osmotic pressure is considered as a pressure exerted by the dissolved substance toward the outside upon the confining walls and the free surface. Ordinarily this pressure never becomes evident on the outside, because it is always much surpassed by the reverse action of the surface tension of the fluid. When the surface tension is removed, as is the case when the solution is separated from the pure solvent by a semipermeable membrane, then the osmotic pressure becomes evident, as either the membrane is moved by the pressure, or in case the membrane is not movable and is not ruptured, pure solvent enters into the solution. The pressure of the surface tension, which is transferred through the membrane, is less than the sum of the surface tension of the solution and the reverse-acting osmotic pressure; and the solvent is, therefore, pressed against the membrane, with a force equal to the osmotic pressure of the solution.

For physiological purposes it seems best to make use of the above explanation, according to which the osmotic pressure is considered as a measure of the force with which a solution attracts the solvent.

PFEFFER'S above-described method of directly determining the pressure can only be used in exceptional cases, first because the preparation of the semipermeable membrane is connected with difficulties, and second because there are only a few bodies which have been found impermeable to the membranes. There are other quicker and easier ways of determining the osmotic pressure.

Solutions of non-volatile substances boil at a higher temperature than the pure solvent. This is due to the fact that the dissolved substance, because of the osmotic pressure, holds on to the solvent with a certain force. As in boiling a part of the solvent is separated from the dissolved body and as the osmotic pressure can be considered as a measure of the attractive power between the solvent and the dissolved substance, then it is clear that solutions which are prepared with the same solvent and have the same osmotic pressure (isosmotic solutions) must also boil at the same temperature. The rise in the boiling-point of a solution above the boiling-point of the solvent (elevation of the boiling-point) is also, like the osmotic pressure, for dilute solutions proportional to the concentration.

Solutions have a lower freezing-point than the pure solvent, and as in dilute solutions the solvent can be frozen out from the dissolved body, then isosmotic solutions have the same freezing-point. The depression of the freezing-point is also proportional to the concentration.

The determination of the elevation of the boiling-point for the estimation of the osmotic pressure of animal fluids is applicable only in exceptional cases, because on heating, precipitates often form. The determination of the depression of the freezing-point has been found of much greater use. This can be accomplished in a easy manner by aid of the apparatus suggested by BECKMANN. In regard to the use of this method we must refer to more complete works.[1]

The above rule that equimolecular solutions of different bodies have the same osmotic pressure is only applicable to non-electrolytes. The electrolytes (bases, acids, salts) show in aqueous solution a much greater pressure (i.e., a much lower depression of the freezing-point) than equimolecular solutions of non-electrolytes. As is known, ARRHENIUS has explained this lack of correspondence by the assumption that the molecule of the electrolyte is divided or dissociated into so-called ions having an opposed electric charge. An ion exerts upon the osmotic pressure the same influence as the non-dissociated molecule. The larger the number of dissociated molecules the more does the osmotic pressure of the solution rise above the pressure of an equimolecular solution of a non-dissociated body. The osmotic action of a dissociated body is equal to that of a non-dissociated body which in a given volume contains as many molecules as the dissociated body contains ions plus non-dissociated molecules. If we assume that a is the degree of dissociation, i.e., the number of the molecules that are dissociated, then $1-a$ is the number that is not dissociated. If in the dissociation of a molecule n ions are formed, then the relation of the molecules present before the dissociation to the ions + molecules present after the dissociation is $1:(1-a+na)$ or $=1:(1+[n-1]a)$. The expression $(1+[n-1]a)$ is generally denoted by the letter i, and can be directly determined by estimating the freezing-point of a solution of known molecular concentration.

A gram-molecule aqueous solution (one that contains as many grams per liter as the molecular weight of the substance) of any non-electrolyte freezes at about $-1.86°$, or, the depression of the freezing-point Δ is $=1.86°$. For example, if we find that Δ for a gram molecular solution of NaCl is 3.40° then we have according to the above $1:(1+[n-1]a)=1.86:3.40$. In the dissociation of NaCl two ions are formed, therefore $n=2$, and from the above equation the degree of dissociation can be calculated, $a=0.83$. The degree of dissociation can also be calculated from the electrical conductivity. Only the ions take part in the con-

[1] Fuchs, Anleitung zu Molekulargewichtsbestimmungen. Leipzig, 1895; Ostwald-Luther, Hand- und Hilfsbuch zur Ausführung physik.-chemischer Messung, 2. Aufl., 1902.

duction of electricity, and the molecular conductivity $\left(=\frac{\text{conductivity}}{\text{molecular concentration}}\right)$ is proportional to the degree of dissociation. The dissociation increases with the dilution and at infinite dilution all molecules are dissociated ($a=1$). If we designate with μ_∞ the limit value which the molecular conductivity approaches in infinite dilution and with μ_ν the molecular conductivity at some definite dilution ν, then the degree of dissociation at this dilution is $a=\frac{\mu_\nu}{\mu_\infty}$.

The positively charged ions are called cations, and the negatively charged anions. Common for all acids are the positively charged H-ions while the negatively charged OH-ions are common for all bases.

Osmotic Experiments with Plant Cells. We often meet the word *osmosis* in literature without understanding exactly what is meant thereby. As a rule *diffusion streams* are meant, which are modified by means of the permeability conditions of an inclosing membrane. We now know that the driving force, namely, the streaming, is brought about by the differences in concentration, i.e., by difference in the osmotic pressure on the two sides of the membrane.

After NÄGELI found that certain plant cells, when they were treated with a sufficiently concentrated solution of certain bodies, changed their appearance so that the protoplasm retracted,[1] DE VRIES studied this phenomenon further.[2] This phenomenon is called *plasmolysis* by DE VRIES. The most important substances for bringing about plasmolysis are the salts of the alkalies and alkaline earths, varieties of sugars, monatomic alcohols, and neutral amino-acids. An indispensable condition for bringing about plasmolysis is that the solution must not have any destructive action upon the cells. NÄGELI gave the correct interpretation of plasmolysis, which is that those bodies which plasmolyze plant cells pass through the cell membrane of the cell, but not through the protoplasmic layer which follows. Instead of this the substance attracts water from the inner parts of the cell. The cell contents surrounded by protoplasm therefore diminish in volume and the protoplasm recedes more or less from the cell membrane. From this it follows that only those solutions whose power of attracting water is greater than that of the cell contents can bring about plasmolysis. As the ability to attract water (or the osmotic pressure) increases with concentration, there must be a limit solution for every substance above which all higher concentrations plasmolyze. The limit solution is called *isotonic* with the cells; weaker solutions are called *hypotonic*, and stronger *hypertonic*. DE VRIES, with the aid of equal cells (cells of the epidermis of the lower side of the leaf of the Tradescantia discolor) has, for various substances, determined the concentration of this limit solution. It

[1] Pflanzenphysiol. Untersuch., 1855.

[2] Eine Analyse der Turgorkraft, Jahresber. f. Wissensch. Botanik, **14**, 427, 1884.

was found that the limit solution of analogously constructed salts had the same molecular concentration. Thus the alkali salts of the type NaCl (haloid salts, nitrate, acetate) plasmolyzed at one molecular concentration and the salts of the type Na_2SO_4 (sulphate, oxalate, diphosphate, tartrate) at another concentration. If the plasmolyzing power of a molecule of the first group is equal to 3, then the molecule of the second group equals 4. The concentration of the limit solution varied in DE VRIES' experiments between the limits corresponding to a NaCl solution of 0.6–1.3 per cent.

As above mentioned, only those substances bring about plasmolysis which cannot themselves pass through the protoplasm envelope of the cell content, and these substances only in the case that the concentration is sufficient. If a body is taken up by the protoplasm it produces no plasmolysis, because its tendency to attract water has been satisfied by its own passage into the cell. These substances do not produce plasmolysis in any concentration. If a body slowly passes in, then at first it causes plasmolysis, but this then ceases later. The plasmolytic methods have been used by DE VRIES, and especially by OVERTON.[1]

Experiments with Blood Corpuscles. Over a hundred years ago HEWSON observed that the blood corpuscles were destroyed in water, and that salts in certain concentrations prevented destruction.[2] HAMBURGER[3] has carefully and systematically investigated the action of salts of the alkalies and alkaline earths, and concludes that when blood is mixed with certain volumes of solutions of different concentrations of the same salt, all solutions whose concentration lies below a certain limit cause the exudation of hæmoglobin. On comparing the molecular concentration of the limit solution of different salts it was found that they bore the same relation to each other as the relative figures found by DE VRIES for the osmotic action. From this it probably follows that the protective action of the salts upon the blood corpuscles depends upon the same reasons as the plasmolysis. This conclusion is also supported by the fact that those substances which, according to DE VRIES, in proper concentration cause plasmolysis in living plant cells, can also under similar conditions prevent the exudation of hæmoglobin. Those bodies, on the contrary, which do not cause plasmolysis, act in aqueous solution in the same manner upon the blood corpuscles as pure water. This has been especially shown by the investigations of GRYNS.[4]

Different investigators have attempted to perform plasmolytic

[1] Vierteljahrschr. d. Naturf. Gesellsch. zu Zurich, **40**, 1, 1895; **41**, 383, 1896.
[2] Phil. Trans., 1773, p. 303.
[3] Arch. f. (Anat. u.) Physiol., 1887, p. 31; Zeitschr. f. Biol., **26**, 414, 1889.
[4] Pflüger's Arch., **63**, 86, 1896.

experiments with animal cells, but without any special result. With the microscope one can often observe that the red blood corpuscles shrink under the influence of a strong salt solution, but the limit solution when the shrinking just begins cannot be exactly determined because the changes in volume are so very small. If we summate the changes in volume of many corpuscles, which can be done by centrifuging the blood mixture in a graduated tube, then very small changes can be detected. Such determinations have been made by HEDIN,[1] KOEPPE[2] and others. It was found that the blood corpuscles swell in a weak salt solution and shrink in a stronger solution and there is a certain concentration which does not change the volume. By determining the freezing-point HEDIN found that this concentration for NaCl was nearly isosmotic with the serum of the blood corpuscles used. The depression of the freezing-point was about 0.56° and the concentration of the NaCl solution is 0.9 per cent, or about 0.15 normal.

The question as to the permeability of the blood corpuscles has been investigated by HEDIN, using a method depending upon the following:[3]

The depression of the freezing-point of a solution is proportional to its concentration. A certain amount of the substance to be tested is dissolved in blood. The serum of this treated blood freezes at a lower temperature than before the salt was added. The depression of the freezing-point can be designated as a. Now the same amount of substance is dissolved in serum using the same volume of serum as blood was previously used. The depression of the freezing-point of this serum can be designated as b. From this it is evident that $a=b$ if the blood corpuscles take up just as much dissolved substance from the blood as an equal volume of serum. If the blood corpuscles take up less than the serum then $a>b$ or $\frac{a}{b}>1$, and when they take up more than the serum then $a<b$ or $\frac{a}{b}<1$.

The result $\frac{a}{b}$, in the calculation of which the change taking place in the volume of the blood corpuscles on the addition of the substance must be considered, gives immediately an approximate idea of the quantity of substance which has passed into the blood corpuscles.

The results were as follows:

The salts of the fixed alkalies and alkaline earths, neutral amino acids, varieties of sugars as well as hexatomic and pentatomic alcohols do not pass into the blood corpuscles, or only to a slight degree. Erythrite (tetratomic alcohol), passes slowly, and glycerin (triatomic) also slowly, but faster than erythrite. Ethylene glycol (diatomic alcohol) passes rather rapidly, and the monatomic alcohols divide themselves immediately equally in the serum and blood corpuscles. Ether, esters, aldehyde, and acetone divide themselves so that the blood corpuscles con-

[1] Skand. Arch. f. Physiol., 5, 207, 238, 377, 1895.
[2] Arch. f. (Anat. u.) Physiol., 1895, 154.
[3] Pflüger's Arch., 68, 229, 1897; 70, 525, 1898.

tain more than an equal volume of serum. These bodies are equally absorbed by the blood corpuscles.

OVERTON had previously arrived at the same results, using plant cells, but urea is probably more quickly taken up by the blood corpuscles than by plant cells, and ammonium salts also seem to pass more easily into the blood corpuscles than into the plant cells.

In regard to other salts HEDIN's results have been substantiated by OKER-BLOM,[1] by estimating the electrical conductivity.

It must also be stated that according to HEDIN, only those bodies which do not pass, or pass only slowly into the cells, can essentially alter the volume of the cells. A close correspondence exists in this regard between the plant and animal cells.

GÜRBER found that when blood corpuscles are repeatedly washed with salt solution until the wash solution does not show any alkaline reaction, and are then suspended in NaCl solution and treated with CO_2, the alkaline reaction increased while the blood corpuscles became richer in chlorine. No exchange of K or Na took place.[2] GÜRBER explains the experiment as follows: the carbonic acid set a small amount of HCl free from the salt, and this HCl was taken up by the blood corpuscles. The Na_2CO_3 formed at the same time gave the alkaline reaction to the solution. KOEPPE[3] as well as HAMBURGER and v. LIER[4] claim on the contrary that an exchange of HCO_3-ions and Cl-ions takes place between the blood corpuscles and the solution, and HAMBURGER and v. LIER claim to have shown that the blood corpuscles are permeable only for anions, while the cations do not pass in. GÜRBER's explanation is simpler and stands in accord with the facts as found. The theory as to the permeability for anions does not explain the fact that the anions in the blood corpuscles are so different from those in the plasma.

Muscle Experiments. By investigations on the changes in the weight (instead of the volume changes in the above-mentioned experiments with plant cells and blood corpuscles) which frog muscles undergo in solutions, various experimenters, NASSE,[5] LOEB,[6] and OVERTON,[7] have tried to prove the ability of muscle to take up various substances. OVERTON found that as long as the irritability of the muscle was retained the muscle took up the same bodies as the plant cells. The sarcolemma

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[1] Pflüger's Arch., **81**, 167, 1900.
[2] Sitzungsber. d. med. phys. Gesellsch. zu Würzburg, 1895.
[3] Pflüger's Arch., **67**, 189, 1897.
[4] Arch. f. (Anat. u.) Physiol., 1902, 492.
[5] Pflüger's Arch., **2**, 114, 1869.
[6] *Ibid.*, **69**, 1; **71**, 457, 1898.
[7] *Ibid.*, **92**, 115, 1902; **105**, 176, 1904.

is not responsible for the permeability, but the outer layers of the muscle protoplasm are.

The skin of amphibians seems according to OVERTON to behave like the muscles[1] in regard to permeability

Theories of Admissibility. On what does the permeability or non-permeability of cells for certain bodies depend? The discoverer of precipitation membranes, M. TRAUBE, considered the membrane as a sort of molecular sieve. The relation of the size of the particles passing and the width of the pores of the membrane was important.[2]

OVERTON states that "the reason of the very variable permeability of living protoplasm, or better the plasma membrane for various compounds, lies in an impregnation of the membrane by bodies of similar solvent ability like the high molecular monatomic alcohols, ether, olive oil, etc. All compounds which are readily soluble in this impregnable substance pass quickly into the cell, while those compounds which are considerably less soluble therein than in water pass more slowly into the cell, and indeed the slower the more the distribution coefficient changes in favor of the water (page 48). Those compounds which are practically insoluble in the impregnable substance do not pass into the cell."[3]

Certain substances which are of the very greatest importance for life processes and which probably are burnt to a great extent within the cells, have, according to the above experiments, either no ability to enter the cells, or only a limited ability. These bodies are the sugars and the amino acids. Also the presence of salts within the cells is not easily understood with the above theory. For this reason OVERTON calls the above treated permeability (for which his theory attempts an explanation) *passive*, and differentiates this from the *active*, in which the protoplasm of the cells are active in one unknown way or another.[4]

MOORE and ROAF believe that the salts exist in the blood corpuscles in the form of "adsorpates." The cells therefore do not take up added salts, because the absorbing proteins have reached their saturation limit (p. 48).[5]

Osmotic Pressure of Animal Fluids. As is apparent from the above, a substance exerts upon living cells an entirely different influence, depending upon whether the substance is able to pass into the cell or not, or whether the substance which does not pass in has the ability of attracting water or not. Therefore that part of the osmotic pres-

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[1] Verhandl. d. phys. med. Gesellsch. zu Würzburg, (N. F.), **36**, 277, 1904.

[2] Arch. f. Anat. Physiol. u. Med., 1867, 87.

[3] Vierteljahrsschr. d. naturf. Gesellsch. zu Zurich, **44**, 88, 1899, and Nagel's Handbuch d. Physiol. des Menschen. II, 817.

[4] Nagel's Handbuch d. Physiol. des Menschen. II, 816.

[5] Biochem. Journ., **3**, 55, 1908.

sure of body fluids which is caused by bodies not passing in is called the *effective osmotic pressure.* These bodies include HEIDENHAIN'S lymphagogues of the second order (Chapter VII). Their action, according to HEIDENHAIN, depends upon their ability to abstract water from the tissues. To this group also belongs the salts of the alkalies and alkaline earths and the sugars. As sugar, as well as the bodies which according to the just mentioned experiments are readily taken up by the cells, occurs under ordinary conditions only in very small amounts in the blood, and also as the proteins are practically without influence upon the osmotic pressure, the normal osmotic pressure of the blood is chiefly due to the salts. As the depression of the freezing-point is almost the only method used for animal fluids, therefore ordinarily the freezing-point depression (Δ) is given as a measure of the osmotic pressure. For mammalian blood Δ is constant with the exception of slight variations due to the food and perhaps also to other circumstances. It is 0.56°,[1] which corresponds to a 0.90 per cent NaCl solution and to an osmotic pressure of about 6½ atmospheres. In lower animals Δ may be slightly lower, for example, in the frog $\Delta = 0.46°$. In invertebrate sea animals the body fluid is equal to the osmotic pressure of the surrounding sea water ($\Delta = 2.3°$) and varies with the quantity of salt in the water (BOTTAZZI[2]). In lower fishes (Selachii) the osmotic pressure of the blood is equal to the surrounding medium, and in higher fishes (Teleostomi) lower ($\Delta = 1.0°$) (BOTTAZZI).

In sea fishes as well as fresh-water fishes, for example, the eel, a lower osmotic pressure ($\Delta = 0.41°$) is found when kept in fresh water than when kept in sea water ($\Delta = 0.55°$)[3]. In lower sea animals the osmotic pressure is equal to the surrounding medium, while higher animals are independent of the surroundings. HÖBER calls attention to this condition and points out the analogy with the body heat of the various animals.[4]

If we pass to other body fluids we must mention that the lymph shows a somewhat higher osmotic pressure than the blood, and this is due to the lymph taking up from the tissues metabolic products having a low molecular weight.[5] Milk and bile have the same osmotic pressure as the blood,[6] while saliva has a lower pressure.[7]

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[1] Hamburger, Osmotischer Durck u. Ionenlehre., **1**, 456.

[2] Archives ital. de Biol., **28**, 61, 1897.

[3] Dekhuisen, Arch. néerland, **10**, 121, 1905; Quinton, Compt. rend. soc. biol., **57**, 470, 513, 1904.

[4] Physik. Chem. d. Zelle u. Gewebe, 2. Aufl., 1906, 33.

[5] Leathes, Journ. of Physiol., **19**, 1, 1895.

[6] Dresser, Arch. f. exp. Path. u. Pharm., **29**, 303, 1892.

[7] Nolf, Traveaux du lab. de phys. de Liège, **6**, 225, 1901.

The urine of man and mammalia generally has a much higher osmotic pressure than the corresponding blood.[1] For human urine Δ varies between 1.3 and 2.3°. After abundant drinking as well as under pathological conditions (diabetes insipidus) the osmotic pressure of the urine can be lower than the blood. In regard to the osmotic pressure of animal fluids under normal and pathological conditions we refer to the work of KORÁNYI and RICHTER.[2]

II. COLLOIDS.

The word *colloid* originated with GRAHAM, who included in this name different substances which did not have the property of diffusing through an animal membrane. In opposition to this GRAHAM called those bodies which passed through a membrane, *crystalloids*, because they were as a rule crystalline, a property which with few exceptions does not belong to the colloids.[3] GRAHAM included soluble silicic acid among the colloids and also analogous forms of stannic acid, titanic acid, molybdic acid and tungstic acid, the aluminium hydroxide and analogous metallic oxides, when they exist in the soluble form, and also starch, dextrins, the gums, caramel, tannin, albumin and gelatin.

Some colloids are characterized by the fact that under certain conditions they solidify in a gelatinous form containing considerable water. In the case where water is the solvent then GRAHAM called the soluble form *hydrosol* and the gelatinous form *hydrogel*.

By diffusion through a membrane (called *dialysis* by GRAHAM) colloid substances can be separated from crystalloids. Colloidal silicic acid as well as corresponding forms of certain other bodies are obtained by treating the soluble alkali salt with hydrochloric acid, then removing the excess of hydrochloric acid as well as of chlorides, by means of dialysis. Colloidal alumina was obtained by GRAHAM by dissolving aluminium hydroxide in aluminium chloride. This last salt was removed by dialysis and the hydroxide remained with more or less HCl combined in solution.

Various metallic sulphides can be obtained in colloidal solution. Such solutions of As_2S_3 and Sb_2S_3 can be obtained by passing H_2S into dilute solutions of the respective metallic oxide,[4] and colloidal CuS can be prepared by washing the precipitated compound with water, by which treatment the CuS finally becomes soluble in water.[5]

The metals can be obtained as hydrosols, and indeed in two ways:

1. By treating a salt with various reducing agents (for example formaldehyde, hydrosulphurous acid, hydrazine, hydroxylamine) the various metals are obtained in colloidal solution.[6] As the solutions thus obtained are often very unstable,

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[1] Korányi, Zeitschr. f. klin. Med., **33**, 1, 1897; **34**, 1, 1898.

[2] Physikalische Chemie und Medizin. Leipzig, 1907.

[3] Ann. d. Chem. u. Pharm., **121**, 1, 1862, as well as Ann. de chim. et de Phys. (4), **3**, 127, 1864.

[4] H. Schulze, Journ. prakt. Chem. (N.F.) **25**, 431, 1882 and **27**, 320, 1883.

[5] Spring, Ber. d. d. chem. Gesellsch., **16**, 1142, 1883.

[6] Müller, Allg. Chemie d. Kolloide. Leipzig, 1907, 6.

it has been found advisable to help their stability by the addition of organic colloids (gelatin). We will discuss the mode of action of these so-called protective colloids on page 44.

2. BREDIG [1] has discovered a method which makes possible the obtainment of pure metallic sols by the cathode spraying of metallic wires under water. SVEDBERG [2] prevents the heating of the fluid in this spraying by using the induction current. This makes the spraying also possible under organic fluids and sols of the light metals have also been prepared. Sols of all metals and metalloids can be prepared practically in this way.

Among those bodies which can be obtained in the colloidal state we have acids as well as bases, and the chemical elements are also known as colloids, as well as bodies of more complex molecular structure like the proteins and starches. The colloid bodies, therefore, have from a chemical standpoint nothing in common. More likely the colloid condition is due to physical properties, and this follows from the researches of GRAHAM.

In order to give a better review we will give a classification of the colloids which seems, for the present, to be rather universally accepted. This was first suggested by PERRIN[3] and later accepted by HÖBER,[4] A. MÜLLER,[5] and WO. OSTWALD,[6] although different authors use different names for the two classes. The classifications of HARDY [7] and ZSIGMONDY [8] have also much in common with the classification given below.

One of the two groups of colloids is called *hydrophile colloids* (emulsion colloids, emulsoides) because in the aqueous solution a certain relation still exists between the dissolved substance and the solvent which is evident especially by a certain viscosity of the solution. The hydrophile colloids often gelatinize on cooling, the gel is again soluble in water (reversible), and in general the hydrophile colloids are separated from their solution by electrolytes with greater difficulty than the colloids of the second group. Bodies of the greatest importance for physiological chemistry like the proteins, starch, and glycogen, belong to the hydrophile colloids.

Contrary to the hydrophile colloids the colloids of the colloidal metal type are called *suspension colloids* (suspenoids) as they must be considered as suspended solid particles in a solvent and have no close relation to the solvent. The viscosity of the solution does not differ much from that of the pure solution; besides this, the suspension col-

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[1] Anorganische Fermente. Leipzig, 1901, 24.
[2] Ber. d. d. chem. Gesellsch., **38**, 3616, 1905; **39**, 1705, 1906.
[3] Journ. de Chimie phy., **3**, 84, 1905.
[4] Physik. Chem. d. Zelle u. Gewebe, 2 Aufl., 1906, 208.
[5] Allg. Chemie d. Kolloide, 1907, 187.
[6] Zeitschr. f. Chem. u. Ind. d. Koll., **1**, 331, 1907.
[7] Proc. Roy. Soc., **66**, 95, 1899.
[8] Zur Erkenntnis d. Koll., 1905, 16.

loids do not gelatinize, do not swell up, and are readily precipitated by electrolytes. To this group belong the metallic sols, the colloidal metallic sulphides, and certain typical suspensions obtained by dissolving water-insoluble substances in another liquid (alcohol, acetone) and then pouring this solution into a large volume of water. In this way the substance is precipitated in a finely divided condition. Such suspensions behave in many respects like suspension colloids. Suspensions of mastic,[1] colophony,[2] and cholesterin [3] belong to this class.

The hydrophile colloids stand closer to the crystalloids than to the suspension colloids, and the transition between the crystalloids and the hydrophile colloids is only gradual. At the boundary we find the peptones and proteoses which belong to the proteins, but at the same time dialyze rather well. On the other hand, we also have colloids which to a certain extent form intermediary steps between the hydrophile colloids and suspension colloids. These intermediary members are the colloidal acids and metallic hydroxides, which correspond with the suspension colloids by being readily precipitated by electrolytes. In this case they separate as gels which differ from the gels of the hydrophile colloids by not being again soluble in water. Finally, there are also numerous intermediary members between the suspension colloids and the finely divided substances suspended in water (kaolin).

Osmotic Pressure. As above stated, the osmotic pressure of solutions of crystalloids can be determined only in exceptional cases by means of the semipermeable membrane, because it is very difficult to prepare membranes which are impermeable for crystalloids. As previously stated, most membranes are impermeable for colloids, and the osmotic pressure of the colloids can be best directly determined by the aid of a membrane in a so-called *osmometer*. As shown by Moore and Roaf, in such an apparatus changes in pressure can be determined which are not detectable by the determination of the freezing-point.[4]

Equimolecular solutions of various non-electrolytes give the same osmotic pressure. From this it follows that when different non-electrolytes exist in solutions with the same percentage concentration, the osmotic tension of these solutions must be in inverse proportion to their molecular weights. Certain colloids which will be discussed in another connection (proteins, glycogen, etc.) must have a very large molecule. From this it follows that these bodies must exert a very low osmotic pressure. The proteins always contain a small amount of salts which exist either in a sort of combination with the colloids or are to be considered as contaminations which are difficultly removed.

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[1] Zeitschr. f. physik. Chem., 57, 47, 1906.
[2] *Ibid.*, 38, 385, 1901.
[3] Bioch. Zeitschr., 7, 152, 1908.
[4] Bioch. Journ., 2, 34, 1906.

For this reason it has been repeatedly stated that these salts are responsible for the small differences in the osmotic pressure. By carefully washing crystalline proteins from serum and egg-white, REID was able to prepare bodies which gave finally no osmotic pressure in the osmometer.[1] In opposition to this, MOORE and ROAF as well as LILLIE call attention to the fact that the osmotic pressure of protein solutions is influenced by the treatment which the protein received before the determination. STARLING,[2] MOORE and PARKER,[3] MOORE and ROAF[4] and LILLIE,[5] using protein preparations which had not been exposed to any strong treatment before use (serum proteins, ovalbumin), as well as REID[6] (with hæmoglobin), have been able to detect a low osmotic pressure and indeed by the aid of osmometric methods. According to STARLING, the proteins of the serum correspond to a pressure of 30–40 mm. Hg. and REID found a pressure of 3–4 mm. Hg. for a 1 per cent hæmoglobin solution.

The influence of added bodies upon the osmotic pressure has been tested by LILLIE by adding the substance to be tested in the same percentage concentration to the inner and outer fluids. It was found that non-electrolytes were without action while acid and alkalies increased the osmotic pressure of gelatin solutions, while salts lowered the pressure of gelatin as well as ovalbumin solutions. ADAMSON and ROAF[7] arrived at similar results in regard to alkalies and acids. Besides this, LILLIE found that the osmotic pressure was dependent upon the past history of the colloid. Warming as well as shaking the solutions seems to change the aggregation condition, which returns very slowly or not at all. The changes in the osmotic pressure produced by salts, LILLIE explains by a change in the aggregation condition of the colloid, by the addition of salts it is brought closer to its precipitation point and is probably united in large aggregations which causes a lowering in the osmotic pressure.

Filterability. Large particles suspended in a liquid can be removed from the fluid by filtering. The finer the suspended particles are the thicker must the filter be. Extensive experiments on the filtering of colloids have been carried out by BECHOLD.[8] He used paper filters which were impregnated with collodion dissolved in glacial acetic acid. According to the concentration of the collodion solution filters of different porosity were obtained. The colloid solutions were pressed through the filter by a pressure up to five atmospheres. It was shown that all colloid solutions contained particles of various sizes. Nevertheless for every solution a filter could be prepared whose pores were small enough to retain all the particles. In this manner BECHOLD was able to classify the colloids in a series according to the size of the smallest particles. He found that in general the inorganic colloids (Prussian blue, platinum, iron oxide, gold, silver) form larger particles than the

[1] Journ. of Physiol., **31**, 438, 1904.
[2] *Ibid.*, **19**, 322, 1896.
[3] Amer. Journ. of Physiol., **7**, 261, 1902.
[4] Bioch. Journ., **2**, 34, 1906.
[5] Amer. Journ. of Physiol., **20**, 127, 1907.
[6] Journ. of Physiol., **33**, 12, 1905.
[7] Bioch. Journ., **3**, 422, 1908.
[8] Zeitschr. f. physik. Chem., **60**, 257, 1907.

organic colloids (gelatin, hæmoglobin, seralbumin, proteoses, dextrin). Still it must be remarked that according to ZSIGMONDY[1] the size of the particles of the same colloid are larger in one preparation than in another and that the size can change on keeping.

On filtering proteose solutions through filters of unequal thickness BECHOLD was able to show that the larger the particles of the proteoses, the easier are they precipitable by ammonium sulphate.

Optical Properties. Colloidal solutions are opalescent by reflected light, which depends upon the fact that the light is reflected by the suspended particles. The reflected light is partly polarized. This phenomenon, called TYNDALL'S phenomenon, depends upon the presence of small particles in the liquid, and is considered as a test for colloid solutions. Still there are colloid solutions (certain gold solutions, ZSIGMONDY), which do not give TYNDALL'S phenomenon, and on the other hand we also have solutions of certain high molecular crystalloids (cane sugar, raffinose), which produce this phenomenon.[2]

With the aid of the ultramicroscope of SIEDENTOPF and ZSIGMONDY, it has been made possible to directly see the colloidal particles.[3] In this apparatus the colloidal particles are strongly illuminated by direct light, so that no ray of the light directly falls in the eye of the observer. The particles are hereby made visible on account of the formation of diffraction disks which are visible by the miscroscope. In colloidal solutions where the particles are close together a more or less intense, homogeneous, polarized sphere of light is seen in the microscope where the individual particles cannot be distinguished from each other. This is possible on diluting the solution. Those particles which are only made visible by dilution are called *submicrons*, while those that gradually disappear on dilution are called *amicrons*.

If the quantity of metal and the number of particles are determined in the unit volume of a metallic sol, then from this the size of the particles can be approximately calculated under the assumption that the density of particles is the same as the metal. The *amicrons* must not be considered in such measurements. In this manner the following lineal dimensions have been found for the submicrons of certain metals, bearing in mind that 5 $\mu\mu$ is about the lowest limit of the ultramicroscope for these particles.

Gold.....	6–130 $\mu\mu$[4]
Silver.....	50– 77 "
Platinum	44 "

[1] Zur Erkenntnis d. Koll., 1905, 104, as well as Zeitschr. f. Elektrochem., 12, 631, 1906.

[2] Lobry de Bruyn and Wolff, Rec. trav. chim. des Pays-Bas., 23, 155, 1904.

[3] Zsigmondy, Zur Erkenntniss der Koll. Jena, 1905, 83.

[4] According to Zsigmondy (l.c. page 124) only those gold solutions are stable whose average particles have at least a size of 60 $\mu\mu$. When they are greater than 75 $\mu\mu$ then the particles begin to settle.

The investigations of ZSIGMONDY and others upon the growth of colloidal metallic particles are also interesting. Thus the reduction of gold chloride by formaldehyde, whereby colloid gold is formed, is accelerated by the addition of colloidal gold, and the added particles indeed grow at the cost of the newly reduced gold.[1] In a similar manner the reduction of silver nitrate with ammonia and formaldehyde is helped by the addition of colloidal gold when the reduced silver precipitates upon the gold particles.[2] In such processes the amicrons can enlarge so that they can be observed by the ultramicroscope (submicrons). According to the manner of preparation the colloids may have particles of different sizes. (See page 39).

Submicrons have also been detected in solutions of organic colloids. The work of GATIN-GRUŽEWSKA and BILTZ,[3] who used a specially pure glycogen, must be especially mentioned. They found that the aqueous solution of glycogen contained amicrons as well as easily recognizable submicrons, whose presence was only evident by a homogeneous sphere of light, but on the addition of alcohol conglomerate into detectable submicrons.

Internal Friction. The watery solutions of organic colloids are often characterized by their great thickness or viscosity. In a strictly scientific manner this is expressed by the statement that the internal friction of the questionable solution is great. It seems generally accepted that the internal friction of suspension colloids is equal to that of the pure solvent, or differs from it only slightly. The view is probably correct for various reasons, but as far as purely experimental determinations are concerned it is based upon only a few experiments carried out by FRIEDLÄNDER [4] with colloidal silver and suspensions of colophony. The concentration of the "solutions" in these experiments was low, so that positive conclusions could not be drawn from them.

Molecular Movement. R. BROWN [5] first found that small particles suspended in water showed a quivering motion, and this phenomenon has been called, from its discoverer, Brownian molecular motion. This phenomenon has been observed since then by many investigators in fluids having suspended solid particles as well as in substances dissolved in colloid. It is believed by many that this movement is stopped by the addition of electrolytes.

ZSIGMONDY has found in regard to the molecular movement of colloidal gold that this cannot be caused by a change in concentration due to evaporation, and also not influenced by the duration or intensity of the light applied. Small particles of gold move much more actively than large ones, still sometimes large particles are met which have an active motion. The particles seem to somewhat influence each other, as the activity of the motion generally diminishes on diluting the gold solution. Old gold solutions (several months to 1½ years) may also show active movement.

The molecular movement has been recently studied by SVEDBERG,[6] who used a new method. He has shown that the molecular motion of silver particles is also perfectly normal in an isoelectric point, i.e., for perfectly uncharged particles (page 46). Electrical charge can therefore not be responsible for the molec-

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[1] Zsigmondy, Zeitschr. f. physik. Chem., **56**, 65, 1906.

[2] Zsigmondy and Lottermoser, *ibid.*, **56**, 77, 1906.

[3] Pflüger's Arch., **105**, 115, 1904.

[4] Zeitschr. f. physik. Chem., **38**, 430, 1901.

[5] Edinb. Phil. Journ., **5**, 358, 1828; **8**, 41, 1830.

[6] Studien zu Lehre von den kolloiden Lösungen. Upsala, 1907, 128.

ular movement. SVEDBERG also experimentally proved the previously proposed simple law that the distance traveled in a certain time is in inverse proportion to the viscosity of the means of dispersion. Brownian molecular movement is considered by some as a manifestation of a general molecular movement of matter.

Electrical Transportation of Suspended Particles. A not too weak electric current has the power of causing motion in small quantities of fluid enclosed in a capillary tube or in a porous diaphragm. The particles suspended in a fluid also wander under the influence of the electric current, and indeed to the anode or cathode, according to the nature of the fluid and the particles. This phenomenon is called *cataphoresis*. Such movements have also been found in colloidal solutions. According to BILTZ,[1] in dialyzed aqueous solution, the colloid metallic hydroxides wander to the cathode, and the other colloids (metals, metallic sulphides, acids) wander to the anode. The colloid particles in water are therefore probably electrically charged, hence the negatively charged wander to the anode and the positively charged to the cathode. Dialyzed protein solution shows no cataphoresis. The addition of acid or alkali gives to the protein a positive or negative charge respectively, hence an alkaline solution wanders to the anode and an acid solution to the cathode (HARDY,[2] PAULI [3]).

Precipitation of the Colloids.

The colloids can be separated from their solutions in various ways. Many colloidal solutions are so unstable that they flock out after a time without the addition of anything (silicic acid, metallic hydroxides). Certain colloids appear as flocculent precipitates on heating their solutions (certain proteins, see Chapter III). Others solidify on cooling from hot concentrated solutions, as semisolid forms, so-called jellies or hydrogels, containing considerable water (glue, starch, agar).

On evaporating the hydrosols at ordinary temperature we obtain a residue which ZSIGMONDY divides into reversible and irreversible colloids, according whether they are again soluble in water or not.[4] According to this definition starch, dextrin, agar, gum, protein belong to the reversible colloids while colloidal silicic acid, stannic acid, colloidal metallic hydroxides and sulphides, and the pure colloidal metals belong to the irreversible colloids. The former are relatively non-sensitive toward the addition of electrolytes, while the latter flock out on the addition of the smallest quantity of electrolyte, and indeed again in an irreversible form. This classification stands in accord with what was given above (page 37), as the reversible colloids coincide in a measure with the hydrophile colloids and the irreversible with the suspension colloids.

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[1] Ber. d. d. chem. Gesellsch., **37**, 1095, 1904.
[2] Journ. of Physiol., **24**, 288, 1899.
[3] Hofmeister's Beiträge, **7**, 531, 1906.
[4] Zur Erkenntniss der Koll., page 21.

Electrolyte Precipitation of Suspension Colloids. It must be remarked that for every precipitating electrolyte a certain minimal concentration is necessary to bring about flocking. In comparing the precipitation ability of various electrolytes the concentration of that solution which is just sufficient to cause a visible cloudiness is given in millimolls ($=\frac{1}{1000}$ gram-molecule) per liter.

HARDY[1] has also found that colloids which wander to the anode are chiefly flocked out by the cations of the precipitating electrolyte, and colloids wandering to the cathode are chiefly flocked out by the anions. H. SCHULTZE[2] has proven that the precipitating ability is influenced greatly by the valence of the precipitating ions, as the divalent ions act much stronger than the monovalent and the trivalent are still more active than the divalent. This rule has been substantiated by HARDY and others.[3] This valence rule becomes clear by the following experiment of FREUNDLICH.[4] The figures give the lowest precipitation concentration expressed in millimolls per liter. The hydrosol was As_2S_3 (negative) and the valence of the cations is applicable chiefly for the precipitating action.

$\frac{K_2SO_4}{2}$	65.6	$MgCl_2$	0.717
KCl	49.5	$MgSO_4$	0.810
KNO_3	50.0	$CaCl_2$	0.649
NaCl	51.0	$SrCl_2$	0.635
LiCl	58.4	$BaCl_2$	0.691
$\frac{H_2SO_4}{2}$	30.1	$Ba(NO_3)_2$	0.687
HCl	30.8	$ZnCl_2$	0.685
		$UO_2(NO_3)_2$	0.642
		$AlCl_3$	0.0932
		$Al(NO_3)_3$	0.0982

The precipitating action of anions upon a positive hydrosol ($Fe[OH]_3$) is shown in the following experiment of FREUNDLICH:

KCl	9.03	K_2SO_4	0.204
KNO_3	11.90	H_2SO_4	0.219
NaCl	9.25	$MgSO_4$	0.217
$\frac{BaCl}{2}$	9.64	$K_2Cr_2O_7$	0.194

FREUNDLICH has extended the valence rule by the fact that with a negative sol, H ions, the ions of the heavy metals, as well as organic cations in weaker concentration, have a greater precipitating action than other cations; OH ions as well as organic anions act against the precipitating action of the cations. The reverse is shown with a positive sol; OH ions and organic anions of smaller precipitation concentration than corresponds to their valence; H ions and organic cations act against the precipitating properties of the anions.

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[1] Zeitschr. f. physik. Chem., **33**, 385, 1900.
[2] Journ. prakt. Chem. (2), **25**, 431, 1882.
[3] Proc. Roy. Soc., **66**, 110, 1899.
[4] Zeitschr. f. Chem. u. Ind. d. Koll., **1**, 323, 1907.

Certain above-mentioned suspensions (mastic), as well as other particles suspended in water, act the same as suspension colloids. SCHULZE[1] has found that cloudiness due to clay particles on the addition of clarifying bodies (alum, lime) give a voluminous deposition. SCHLOESSING[2] found that clay suspensions which do not settle after months are precipitated in 24–48 hours by a minimum quantity of lime or magnesia. He also calls attention to the essential role which the salts of sea water must play in the sedimentation of the cloudy fresh water flowing into the sea (delta formation).

In consideration of the conditions just mentioned, under which the suspension colloids are precipitated by electrolytes, the mutual precipitation ability of suspension colloids is of considerable interest. According to what has already been stated, the colloids are considered as carriers of electricity, and it has been proven that the oppositely charged colloids can act precipitatingly upon each other. This rule was first proposed by LINDER and PICTON,[3] and subsequently has been substantiated by many investigators. BILTZ[4] has made especially systematic investigations on this subject and finds that equally charged colloids do not precipitate each other. For the mutual complete precipitation of opposed electrically charged colloids, a certain quantity relation is necessary. On the action of two colloids with opposite charges in variable quantities an optimum of the precipitation action is noticed; while on overstepping the desirable precipitation conditions in both directions no precipitation occurs at all.

In analogy with the mutual precipitation ability of the colloids, BILTZ believes that the especially great ability of most salts of the heavy metals to precipitate colloids lies in the hydrolytically-split and colloid-dissolving metallic hydroxides.

Protective Colloids. Certain hydrophile colloids, which are precipitated with difficulty by electrolytes, have the power of protecting suspension colloids against the precipitating action of electrolytes. MEYER and LOTTERMOSSER[5] have found with silver hydrosol that the presence of protein prevented the flocking out by electrolytes. ZSIGMONDY[6] has investigated the relative action of the protective colloids and has found considerable differences. The figure in milligrams of colloid which is just insufficient to protect 10 cc. of gold solution (0.0053–0.0058 per cent) against the action of 1cc. 10 per cent NaCl solution is called the *gold equivalent* for the respective colloid. Gelatin offers the best protection, then comes isinglass, casein, ovalbumin, gum arabic, Irish moss, dextrin, starch. The colloidal sulphides (As_2S_3, Sb_2S_3, CdS) are also protected in the same manner against the influence of electrolytes (A. MULLER and ARTMANN).[7]

[1] Ann. Phys. (2), **129**, 366, 1866.
[2] Compt. rend., **70**, 1345, 1870.
[3] Journ. chem. Soc., **71**, 572, 1897.
[4] Ber. d. d. chem. Gesellsch., **37**, 1095, 1904.
[5] Journ. prakt. Chem. (2), **56**, 241, 1897.
[6] Zeitschr. analyt. Chem., **40**, 697, 1901.
[7] Oester. Chem. Ztg., **7**, 149, 1904.

Inorganic colloids can also serve as protective colloids. BILTZ[1] has shown that zirconium hydroxide protects gold better than gelatin.

By the addition of organic protective colloids the inorganic colloids which on evaporation otherwise become irreversible, are made reversible, in that the dry residue is soluble in water again. On this depends the use of the protective action in the preparation of permanent inorganic hydrosols, and this is of importance in many cases.

According to BECHHOLD[2] the filterability of suspension colloids through collodion filters is increased by the addition of organic colloids. It is also well known that certain finely divided substances (carbon) pass more easily through a filter in the presence of protein than without protein.

The action of the protective colloids is ordinarily explained according to the theory of QUINCKE[3] on the mutual surface tension of the active bodies, and the process belongs accordingly to the adsorption phenomenon which will be discussed later. According to this theory the protective colloid under certain conditions spreads like an envelope around the particles. In this wise the entire mass takes the properties of the protective colloid and is therefore not precipitated by the electrolyte any more than the protective colloid itself. In filtration the protective colloid acts to a certain extent like a lubricant. This theory of colloid envelope has recently received support by experiments of MICHAELIS and PINCUSSOHN.[4] They found that when suspensions of indophenol and mastic were mixed together the number of particles visible in the ultramicroscope diminished; after mixing, the physical properties of the indophenol (pseudofluorescence, positive cataphoreis) were not evident.

Electrolyte Precipitation of Hydrophile Colloids. The salts of the alkalies precipitate the suspension colloids even in low concentrations. The alkali salts behave differently with the organic colloids. This may in part be due to the fact that hydrophile colloids have much less of a certain electric charge than the suspension colloids. Egg-white by dialysis gradually loses its power of being influenced by the electric current. For this reason the hydrophile colloids are often precipitated from their solution by alkali salt. For this purpose, firstly, certain concentrations are necessary; secondly, the precipitates of the hydrophile colloids are again soluble in water (reversible) in opposition to those of the suspension colloids. In regard to the ability of different alkali salts to act precipitatingly certain laws have been formulated, but they cannot be arranged in a general rule.

On comparing the concentration of various salts just sufficient for precipitation, where at one time the same anion with different cations was tested and

[1] Ber. d. d. Chem. Gesellsch., **35**, 4431, 1902.
[2] Zeitschr. f. physik. Chem., **60**, 301, 1907.
[3] Ann. Phys. (3), **35**, 580, 1888.
[4] Bioch. Zeitschr., **2**, 251, 1907.

another time the same cation with different anions, PAULI has arranged the cations and anions in the following order in increasing precipitation ability:

$$CNS < I < Br < NO_3 < Cl < OCO.CH_3 < HPO_4 < SO_4$$
$$NH_4 < K < Na < Li.$$

The protein used in these experiments was white of egg. According to PAULI certain ions have a precipitating action and others a solvent action. The action of a salt corresponds to the algebraic sum of the action of the ions.[1] PAULI has attempted to associate the precipitation ability of the salts in relation to their action upon the coagulation temperature, but without any positive results.[2]

Nevertheless SPIRO [3] has shown that the kind of protein as well as its concentration are of importance for the precipitation action, and HÖBER[4] has recently shown that the series $I < Br < Cl < SO_4$ and $Li < Na < K < Rb < Cs$ is valid in alkaline reaction, but that the series is reversed in acid reaction. In nearly neutral reaction irregularities in the ion series occur which can be considered as a transition series between the two just-mentioned series. That the reaction must be of great importance in the precipitation of proteins seems very probable in consideration of the fact that the proteins take a decided electric charge on the addition of acid or alkali (see page 42).[5] According to PAULI [6] dialyzed protein, which had no electric charge, could not be precipitated by the addition of salts of Zn, Cu, Hg, Fe, Pb, while the same protein in non-dialyzed form gave heavy precipitates with these. PAULI believes that the native protein of the organism on account of the OH ions originating from the tissue fluids has a negative charge. In regard to the precipitation by salts of the heavy metals, the hydrophile colloids do not seem to differ essentially from the suspension colloids.[7]

Theories of Precipitation Phenomenon.

At least for the suspension colloids there is no question that they are flocked out by ions which carry an electric charge opposite to the colloid particles, and also by other colloids having an opposite charge. This fact follows from HARDY'S theory, according to which the flocking out is a neutralization process in which the charge of the colloid is just neutralized and the colloid therefore precipitates.[8] The mixture formed on precipitation has been shown to be electrically neutral (isoelectric) as the precipitated particles show no cataphoresis. In this manner it is easily understood that polyvalent ions have a stronger precipitating action than monovalent, as the electrical charge in, for example, a trivalent ion is 3 times greater than in a monovalent ion. Otherwise greater precipitation ability of polyvalent ions can also be explained by a greater hydrolytic cleavage of the salts (page 44).

The mechanism of the precipitation of the isoelectric solution accepted in HARDY'S theory is explained by BREDIG [9] as follows: At the boundary between suspended particles and solvent a certain surface tension exists

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[1] Holfmeister's Beiträge, **3**, 225, 1902.
[2] Pflüger's Arch., **78**, 315, 1899.
[3] Hofmeister's Beiträge, **4**, 300, 1903.
[4] *Ibid.*, **11**, 35, 1908.
[5] *Ibid.*, **7**, 531, 1906.
[6] *Ibid.*, **7**, 541, 1906.
[7] *Ibid.*, **6**, 233, 1905.
[8] Zeitschr. f. physik. Chem., **33**, 385, 1900.
[9] Anorganische Fermente, 1901, 15.

which tries to diminish the total contact surface between the two media, which can happen by the small particles uniting to form larger ones, when flocking is brought about. The electrical charge of the particles acts against the surface tension so that equally charged particles repel each other. If the electrical charge is discharged, as takes place in the isoelectric point, then the surface tension reaches its highest value and the precipitation may occur.

The correctness of HARDY'S claim that precipitation occurs just in the isoelectric fluid is disputed on special grounds by BILLITZER. He believes that the ions have a much greater charge than the colloid particles. An ion collects the oppositely charged colloid particles around itself and during these neutralization processes it may occur that the entire complex may become so great that it becomes evident and on account of the gravity it precipitates out.

In general it can be stated that the stability of a colloid is greater the smaller, *cet. par.*, the particles are; as the probability that the number of particles sufficient for the precipitation is then less. With equal size of particles the stability of a colloid is dependent upon the size of the charge which the particles carry. Too weak and very strongly charged colloids are relatively more stable; the first because of the large number which must collect around an ion when flocking takes place and the second because the number of particles required for the neutralization is perhaps too small, so that the necessary size of the complex for precipitation is not attained.[1]

The findings of LINDER and PICTON[2] that when colloidal As_2S_3 is precipitated with $BaCl_2$ the solution becomes acid, and a small quantity of barium remains in the precipitate, corresponds to BILLITZER'S theory. This quantity of barium cannot be removed by water, but can be replaced by the corresponding cation by washing with a solution of another salt. According to BILLITZER in the mutual precipitation of colloids a quantity relation exists which is dependent upon the electrical charges[3] (see also page 44).

The fact that the precipitation of colloids is a manifestation of processes which occur in a homogeneous medium, makes the understanding of these especially difficult. If, as is generally accepted, we consider the colloid solution as a homogeneous fluid of suspended solid or fluid particles, then in the "solution" there occur at least two special constituents, separated from each other—the colloid particles and the solvent. This is expressed as follows: the system contains two *phases*. The solvent is often more correctly called the *dispersion means* and the colloid particles called the *disperse phase*. If to such a system a new

[1] Zeitschr. f. physik. Chem., 45, 327, 1904; 51, 129, 1905.
[2] Journ. Chem. Soc., 67, 63, 1895.
[3] Zeitschr. f. physik. Chem., 51, 141, 1905.

substance is added, then the reaction which follows depends essentially upon the division of the new substance between the two phases. In regard to the possible division two cases will be presented:

1. The process can be similar to the division of a soluble substance between two solvents. If a substance is brought in contact with two solvents at the same time, then it divides itself so that the relation between the concentration in the two solvents remains the same but independent of the total quantity of the dissolved substance. If the quantity of substance in each 100 cc. of the two solutions 1 and 2 is designated by c_1 and c_2, then it follows that $\frac{c_1}{c_2}=k$ where k is a constant.[1] The first example where this law was shown to be correct was the division of succinic acid between water and ether (BERTHELOT and JUNGFLEISCH[2]). This law was also shown to be true for the division of a gas between a gaseous and a fluid phase, i.e., for the absorption of a gas in a fluid (HENRY'S law of absorption). The conditions for the correctness of this law are that the temperature remains the same in experiments with different quantities of substance as well as that the substance has the same molecular size in the two phases.

2. In those cases where finely divided solids take up dissolved substances or gases the division is generally not independent of the total quantity of the dissolved substance or of the gas. For example, if we are dealing with the absorption of a dissolved substance by a finely divided solid occurring in a solution, then a greater percentage is taken up from a dilute solution than from a concentrated one. On increasing concentration the absorbed fraction becomes continuously less so that the absolute quantity taken up reaches a maximum which corresponds to the greatest absorption ability of the solid body. This is expressed by the formula $\frac{{c_1}^n}{c_2}=k$, where c_1 and c_2 indicate the concentration of the solid body and in the solution; n and k are constants and indeed, n is always >1. (If $n=1$ then the formula would be $\frac{c_1}{c_2}=k$ and we would be dealing with a so-called solid solution). The process here treated is called *adsorption*.[3]

APPLEYARD and WALKER[4] have studied the adsorption of organic acids from aqueous and alcoholic solutions by means of silk; the divi-

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[1] Nernst, Zeitschr. f. physik. Chem., 8, 110, 1891.

[2] Ann. Chim. phys. (4), 26, 396, 1872.

[3] It must be remarked that in the older literature oftentimes no difference was made between adsorption, and absorption, in which case both processes were included under the name absorption.

[4] Journ. Chem. Soc., 69, 1334, 1896.

sion was found to correspond to the above formula for adsorption. Recently FREUNDLICH[1] has carefully tested the adsorption of crystalloids by carbon. From these experiments it was shown that the equilibrium could be quickly attained from both sides, i.e., that the process was readily reversible. The above-given formula was found sufficiently accurate for the case where only the total quantity of the dissolved (to adsorb) substance varied. The series in which the organic acids were adsorbed by silk, as found by APPLEYARD and WALKER, were practically the same as with carbon. The influence of temperature was slight.

According to KÜSTER,[2] the combination between starch and iodine is to be considered as an adsorption compound, and BILTZ[3] finds for the division of As_2O_3 between iron hydroxide (1) and water (2) the formula $\frac{c_1}{c_2} = 0.631$.

The theoretical foundations for the adsorption phenomenon are not especially clear. Generally the adsorption is considered as connected with segregation and surface tension phenomenon. At the contact surface between a solid body and solution a surface tension exists which is considered as positive, i.e., the same attempts to diminish the contact surface. The surface energy used thereby tends to be a minimum potential energy. As the product from size of surface and surface tension are the same, and as the first cannot change, the surface energy can only be diminished by a reduction of the tension. If, therefore, the tension is diminished by increasing the concentration of a substance dissolved in a fluid, then this substance tries to collect itself at the surface in greater concentration than in other parts of the fluid (OSTWALD,[4] FREUNDLICH[5]). In regard to the surface tension of solid-fluid we only know that it is positive, but can otherwise show great differences (OSTWALD,[6] HULETT[7]). According to this theory the facts are that certain solid substances possess the ability of adsorbing dissolved bodies, and for this reason the adsorbed substance lowers the surface tension of the solid-fluid, and indeed, the more the greater concentration in which it occurs. That especially carbon and colloid substances are adsorption bodies lies in the fact that they have an especially large surface due to their finely divided state or porosity, which therefore, *cet. par.*, must give then a great surface energy.

[1] Ueber die Adsorption in Lösungen, Leipzig, 1906.
[2] Ann. d. Chem. u. Pharm., **283**, 360, 1894.
[3] Ber. d. d. chem. Gesellsch., **37**, 3138, 1904.
[4] Lehrb. d. allg. Chem., 2. Aufl., 2. Bd., 3. Teil, **237**, 1906.
[5] Ueber Adsorption in Lösungen, 50–51.
[6] Zeitschr. f. physik. Chem., **34**, 495, 1900.
[7] *Ibid.*, **37**, 385, 1901.

That proteins, on precipitation, carry down other bodies with avidity is well known; inorganic hydrogels also take up dissolved substances with energy. The curves obtained for the latter process by VAN BEMMELEN [1] show a close analogy with the characteristic curves for the adsorption compounds. It often occurs that the body taken up homogeneously saturates the hydrogel, in which case $\frac{c_1}{c_2} = k$, and a sort of solid solution is the result. In this manner KCl is taken up by colloidal silicic acid.[2] In certain cases undoubtedly chemical combinations with quite positive conditions are formed.[3]

The precipitation of colloids by electrolytes has also been discussed by FREUNDLICH [4] from the standpoint of the adsorption hypothesis. Thus, for the precipitation ability of an electrolyte, the electric charge of the precipitating ion comes first in consideration and secondly, the ability of the precipitating colloid to adsorb the same. According to MOORE and ROAF [5] the salts of the red corpuscles are retained as adsorption compounds (adsorpates) by the proteins.

Thus far only the adsorption of crystalloids has been considered. Colloids are also taken up by solid substances or by other colloids. Still in these cases the conditions are more complicated than in the above-mentioned adsorption phenomena, as the combinations formed are in special cases irreversible or gradually irreversible. It is well known that carbon takes up colloidal colored substances, and we have numerous examples of the combination of dissolved colloids with solid colloids in technology. BILTZ [6] has been able to show that many dyeing processes are to be considered as adsorption phenomena, and later FREUNDLICH and LOSEV [7] have measured the adsorption of basic and acid pigments by carbon and also by fibers (wool, silk, cotton), and have shown the correspondence of the two processes. With the basic pigments, which were used as salts, a splitting occurred into a pigment base, which was taken up by the fibers as well as by carbon, and an acid which quantitatively remained behind. This is similar to the cleavage which precipitating electrolytes undergo in the precipitation of the suspension colloids (see page 47).

Tanning is also brought about by adsorption processes as the prepared skins adsorb the tanning substance.[8]

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[1] Zeitschr. anorg. Chem., **23**, 111, 321, 1900.
[2] Schmidt, Zeitschr. f. physik. Chem., **15**, 56, 1894.
[3] v. Bemmelen, Journ. prakt. Chem. (2), **23**, 324 and 379, 1880.
[4] Zeitschr. f. Chem. u. Ind. d. Koll., **1**, 321, 1907.
[5] Bioch. Journ., **3**, 55, 1908.
[6] Ber. d. d. chem. Gesellsch., **37**, 1766, 1904; **38**, 2963, 2973, 4143, 1905.
[7] Zeitschr. f. physik. Chem., **59**, 284, 1907.
[8] See Zeitschr. f. Chem. u. Ind. d. Koll., **2**, 257, 1908.

The precipitation of protein by adding finely divided solids (carbon, kaolin [1]) or by suspended solids (mastic [2]) precipitated in the liquid, is also due to adsorption processes. The precipitation of protein, which occurs on shaking the protein solution with liquids, in which the protein is not soluble, is also to be considered as a surface tension action (RAMSDEN).[3]

BECHOLD,[4] in his above-mentioned experiments on the filtration of colloids, has observed conditions which he considers as adsorption phenomena. Under certain circumstances a colloid can prevent the filtration of another colloid. A filter which was permeable for colloidal As_2S_3, but retained colloidal Prussian blue, did not allow a clear mixture of the two to pass through. The particles of As_2S_3, were adsorbed by the particles of Prussian blue, and could therefore not pass through the filter.

Especially with regard to the precipitation of hydrophile colloids we must mention another theory suggested by SPIRO.[5] According to this the precipitation depends upon a division of the colloid between two phases, one of which contains considerable water and salt and little colloid and the other much colloid, but little water and salt.

Gels. We have already often mentioned gels or jellies (page 36). Only certain colloids can occur in the form of gels. Certain gels are spontaneously formed in sufficiently concentrated solutions (silicic acid, certain metallic hydroxides) and these do not redissolve in water. Other gels, like gelatin and agar, are formed on cooling of the hot, concentrated solutions, and are again soluble in water.

According to HARDY [6] the gel formation of gelatin is to be considered as a segregation process whereby a separation into two fluids occurs, one of which solidifies. The two phases are only differentiated by the microscope, and the chemical testing of the theory fails because of the circumstances that the two phases cannot be analyzed separately.

When gels are freed from water by evaporation or in other ways, they show a special ability to take up water which is brought about by different processes which are included in the ordinary term imbibition. The views on this imbibition are indefinite. Surface phenomena play a role here. According to VAN BEMMELEN [7] the water is not chemically combined in definite proportions, but the quantity continually changes with the temperature and the vapor pressure. On the other

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[1] Bioch. Zeitschr., **5**, 365, 1907.
[2] *Ibid.*, **2**, 219, 1906; **3**, 109, 1906.
[3] Zeitschr. f. physik. Chem., **47**, 343, 1904.
[4] *Ibid.*, **60**, 299, 1907.
[5] Hofmeister's Beiträge, **4**, 300, 1903.
[6] Zeitschr. f. physik. Chem., **33**, 326, 1900.
[7] Zeitschr. anorg. Chem., **13**, 233, 1896; **20**, 185, 1899.

hand, the imbibition stands in close relation to the osmotic pressure which is evident, if we define the osmotic pressure of a substance as its ability to attract water. The relation between imbibition and osmotic pressure is still closer in those cases when the substance finally is dissolved in water.

If a hydrogel is placed in a salt solution instead of in pure water, the imbibition phenomena essentially change. This was first studied by HOFMEISTER,[1] using gelatin plates. The process is rather complicated, as salt is taken up by one side of the gelatin plate and water by the other, and the taking up of water is influenced by the quantity of salt taken up. It has been recently found that when gelatin plates are treated with solutions of increasing concentration of the same salt, the taking up of salt increases at first with the salt concentration, then becomes slower, and attempts to reach a maximum and then remains almost stationary. As long as the taking up of salt increases, the quantity of water passing into the gelatin also increases; when the salt fails to pass then the water also ceases to pass. It has also been found that the maximum of salt absorption for sulphate, tartrate and citrate can be attained with much lower molecular concentrations than with chloride, nitrate and bromide. From this it follows that the sulphate, tartrate and citrate have a retarding action upon imbibition within certain limits of concentration, while the chloride, nitrate and bromide have an accelerating action.

PAULI[2] has investigated the influence of salt solutions upon the solidification and melting-point of gelatin. If the salts are arranged in the order of their ability to lower the solidification point of gelatin we come to the series sulphate, citrate, tartrate, acetate (water), chloride, chlorate, nitrate, bromide, iodide. This series corresponds well with that of HOFMEISTER.

Acids and alkalies exert a special influence upon gelatin, as they both in very dilute solutions strongly accelerate imbibition (SPIRO,[3] WO. OSTWALD[4]). From the previously mentioned investigations of LILLIE, on the osmotic tension of gelatin solutions, it was found that the addition of acids and alkalies increased it (page 39).

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[1] Arch. f. exp. Pathol. u. Pharm., **28**, 210, 1891.
[2] Pflüger's Arch., **71**, 333, 1898.
[3] Hofmeister's Beiträge, **5**, 276, 1904.
[4] Pflüger's Arch., **108**, 563, 1905.

III. CATALYSIS.

When two bodies which can act chemically upon each other are brought together the reaction generally takes place so fast that it cannot be measured. In other cases, by special means, we can observe how the reaction gradually proceeds. When cane sugar is inverted by weak acid, the decrease in the rotation of the solution can be followed with the polariscope; and when an ester is decomposed by alkali the quantity of still free alkali can be determined by titration. The quantity of substance measured in gram-molecule per liter (mole) which is decomposed in the unit of time, is called the *reaction velocity* of the system. The so-called law of *mass action*, as proposed by GULDBERG and WAAGE, states that the reaction velocity is every moment proportional to the molecular concentration of the reacting bodies. A mixture of alcohol and acetic acid is transformed into acetic ether and water, especially in the presence of some mineral acid. If the molecular concentration of the alcohol and acid be designated by C_A and C_S, then according to the law of mass action the reaction velocity is $v_1 = k_1.C_A.C_S$, where k_1, indicates a constant which is independent of the quantity of reacting substances and the time limit is so short that the concentration can be considered as constant. This reaction, like many others, is reversible, i.e., two reactions occur simultaneously: one between the alcohol and acetic acid, producing acetic ether and water, and second, between acetic ether and water, reforming alcohol and acetic acid. This is expressed as follows:

$$C_2H_5.OH + HO.CO.CH_3 \rightleftarrows C_2H_5.O.CO.CH_3 + H_2O.$$

The velocity of reaction when it passes from left to right is called v_1. If the velocity in the reverse reaction is called v_2 and the molecular concentration of the acetic ether and water is called C_E and C_W, then we obtain $v_2 = k_2.C_E.C_W$. At the beginning when C_E as well as $C_W = 0$, the velocity of the ester formation is expressed by the formula $v_1 = k_1.C_A.C_S$; afterward it is expressed by the difference $v_1 - v_2$ or $k_1.C_A.C_S - k_2.C_E.C_W$. When $k_1.C_A.C_S = k_2.C_E.C_W$ is attained, then the velocity of both reactions is the same; no measurable decomposition occurs and the system is in equilibrium. The equilibrium condition is the same irrespective of whether we start from alcohol + acetic acid or from the corresponding quantity of acetic ether + water. On equilibrium it is

$$k_1.C_A.C_S = k_2.C_E.C_W \text{ or } \frac{C_A.C_S}{C_E.C_W} = \frac{k_2}{k_1} = K.$$

K is called the equilibrium constant; as is apparent it can be determined in two ways—either from the concentration of the reacting bodies when

equilibrium is present or from the velocity coefficient k_1 and k_2 as determined in a manner given below.

In the above-mentioned transformation of alcohol and acetic acid these two bodies are simultaneously used up. The reaction is therefore called bimolecular, and a reaction is called mono-, bi-, tri-, etc., molecular according to the number of the kinds of molecules which diminish their concentration thereby.[1]

BERZELIUS[2] found that certain bodies by their mere presence, and not by their affinity, have the power of awakening the dormant affinity at a certain temperature. These phenomena were called *catalytic* by BERZELIUS.

According to OSTWALD[3] catalysis is the acceleration (or retardation) of a slow-proceeding chemical change by the presence of a foreign body. That body which influences a reaction in this manner is called a *catalyst.* It does not undergo any appreciable change by the reaction.

Catalytic reactions have been studied, especially by WILHELMY,[4] VAN'T HOFF,[5] OSTWALD,[6] ARRHENIUS[7] and BREDIG.[8] Of all other substances the acids and alkalies seem to act most catalytic. A well-known example is the inversion of cane sugar by means of acid. This reaction is monomolecular because only the cane sugar is consumed. If the concentration of the cane-sugar at the beginning is C moles, and if x moles are transformed in t time, then at that time there are $(C-x)$ moles remaining. If dx indicates the quantity which is transformed in dt time, then the reaction velocity is $\frac{dx}{dt}$. According to the law of mass action this is at every moment proportional to the concentration of the decomposing substance, or

$$\frac{dx}{dt}=k.(C-x). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

For practical use this equation is integrated into the following:

$$k=\frac{1}{t}\text{ nat. log. }\frac{C}{C-x}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

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[1] It is assumed here that of every kind of molecule one molecule of each takes part in the reaction.

[2] Berzelius, Årsberättelse om framstegen i Fysik och Kemi., **13**, p. 245, 1836.

[3] Lehrb. d. allg. chem. 2. Aufl. II., **1**, 515.

[4] Poggendorff's Ann., **81**, 413, 1850.

[5] Études de dynam. chim., 1884.

[6] Lehrb. d. allg. Chem., 2. Auf. II, **2**, 199.

[7] Zeitschr. f. physik. Chem., **4**, 228, 1889.

[8] Anorganische Fermente, 1901; Bioch. Zeitschr., **6**, 283, 1907.

If the theoretical considerations upon which this formula is based are correct, then the x values determined by the polariscope after various times must give the same figure for k. This is indeed the case.[1] k is called the *velocity coefficient* (also velocity constant or specific reaction velocity). If in the equation (1) $C-x$ or the concentration of the still undecomposed cane-sugar $=1$, then the equation becomes $\frac{dx}{dt}=k$, from which it follows that k indicates the reaction velocity if the concentration of the substrate could be kept the entire time at $=1$.

In these experiments k retains the same value. If in different experiments the quantity of catalyst (acid) varies, then the obtained value for k is proportional to the concentration of the H ions. This is so prominent that the catalytic action of acids is due to the H ions (ARRHENIUS [2]). Still irregularities occur as the anions of acids as well as of salts present can under certain circumstances influence the action of H ions (see page 73).

FRÄNKEL [3] has recently studied the decomposition of diazoacetic ether under the influence of different acids. The reaction is as follows:

$$N_2:HC.CO.O.C_2H_5+H_2O=HO.CH_2.CO.O.C_2H_5+N_2.$$

The progress of the reaction can be determined by measuring the nitrogen set free. The following figures explain the results:

Acid.	Conc. of the Acid in Mol. per Liter.	C_H. Conc. of the H-ions by Electric Conductivity.	K Velocity Coefficient.	$\frac{K}{C_H}$
Nitric acid	0.001820	0.001820	0.0703	38.7
	0.000909	0.000909	0.0346	38.0
Picric acid	0.000909	0.000909	0.0356	39.2
	0.000364	0.000364	0.0140	38.3
m-Nitrobenzoic acid	0.009900	0.001680	0.0632	37.7
Fumaric acid	0.003640	0.001460	0.0571	39.1
Succinic acid	0.009090	0.000724	0.0285	38.5
Acetic acid	0.018200	0.000563	0.0218	38.7

As $\frac{K}{C_H}$ for the different acids and different quantities of acid is the same, then the velocity coefficient is here also proportional to the concentration of the H ions.

As the catalytic action of acids is caused by the H ions, so are the catalytic properties of bases due to the OH ions. The first determined case of this kind was the transformation of hyoscyamine into the stable atropine.[4]

[1] See Poggend. Ann., **81**, 413 and 499, 1850.
[2] Zeitschr. f. physik. Chem., **4**, 226, 1889.
[3] *Ibid.*, **60**, 202, 1907.
[4] Ber. d. d. chem. Gesellsch., **21**, 2777, 1888.

KOELICHEN[1] has studied a specially pretty case of the catalytic action of OH ions in the decomposition of diacetonalcohol into acetone:

$$CH_3.CO.CH_2.C(CH_3)_2.OH = 2CH_3.CO.CH_3.$$

The reaction is reversible, and from the following table it is seen that the velocity constant for various concentrations of the same catalyst remains the same as well as by using different bases.

Catalyst.	Conc. of the Catalyst.	Velocity Constant.
Piperidine	0.1090	0.038
Triethylamine	0.4900	0.036
Ammonia	0.5500	0.038
Tetraethylammonium hydroxide	0.0760	0.037
	0.0076	0.037
Sodium hydroxide	0.0725	0.036
	0.0072	0.035

By this a rule which VAN'T HOFF and OSTWALD[2] proved by thermodynamic means, is substantiated, namely, that the equilibrium at constant temperature does not change with the quantity and kind of catalyst when the catalyst is not changed by the reaction.

Among other kinds of ions which act as catalysts we must mention (1) iodine ions, which decompose H_2O_2 in proportion to their concentration,[3] and (2) cyan-ions, which transform benzaldehyde into benzoin according to the following equation:

$$2C_6H_5.COH = C_6H_5.CO.CH(OH).C_6H_5.$$[4]

The above-treated catalytic processes all occur in homogeneous systems, i.e., the systems which by mechanical means cannot be separated into different constituents. In heterogeneous systems with phases which can be separated from each other by mechanical means, catalytic reactions can also occur, and indeed, in such cases the substances taking part in the reaction and the catalyst occur in different phases. Such a reaction is the union of detonating gas, the synthesis of SO_3 (from SO_2 and O), and the decomposition of H_2O_2 by platinum. In case the system is two-phased, and the reaction takes place only at the boundary between both phases, or in the one we can differentiate two simple limits:

1. The accumulation of the bodies which are necessary for the reaction at the proper place takes such a short time that in comparison with the real chemical reaction it can be neglected. In these cases the reaction velocity behaves similarly to a homogeneous system.[5]

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[1] Zeitschr. f. physik. Chem., **33**, 129, 1900.

[2] Van't Hoff, Vorlesungen, **1**, 211.

[3] Walton, Zeitschr. f. physik. Chem., **47**, 185, 1904.

[4] Stern, *ibid.*, **50**, 513, 1905.

[5] Goldschmidt, Zeitschr. f. physik. Chem., **31**, 235, 1899.

2. The chemical reaction occurs at a rate which in comparison with the time necessary for the accumulation can be neglected. In this case the time necessary can be generally compared with a diffusion process.[1]

The catalytic processes in heterogeneous systems have excited interest since BREDIG [2] showed that the colloidal metals prepared by him showed catalytic properties. The best-studied process is the decomposition of H_2O_2 by colloidal platinum, gold, and other metals or oxides (MnO_2, PbO_2). Attention must be called to the small quantity of catalyst sufficient to decompose H_2O_2. The action of 1 gram atom platinum in 70 million liters of reaction mixture has been detected. The decomposition of H_2O_2 by platinum catalyst in nearly neutral or faintly acid solution has been shown to be a monomolecular reaction.

In this connection we will give one experiment of BREDIG and v. BERNECK: [3]

Time.	Conc. of H_2O_2.	$0.4343k$.
0	47.4	
10	37.9	0.0097
20	30.0	0.0099
30	23.6	0.0101
40	18.2	0.0104
60	11.0	0.0106

Still certain differences occur from the conditions formed in the homogeneous catalysis. At one time in certain experiments the value for k rises considerably during the catalysis, and secondly, k is not proportional to the ferment concentration, but rises more quickly than this.

In connection with these experiments BREDIG has expressed the view that an analogy exists between the catalytic processes of the inorganic world and the enzyme action of the organic.

The following important facts give support to BREDIG'S view:

1. In both cases we are dealing with catalytic processes; the metallic sol and the enzyme are active in very small quantities and during the reaction they do not undergo any appreciable change.

2. In the decomposition of H_2O_2 by platinum sols or by the enzyme hæmase, the reaction is monomolecular.

3. The action of metallic sols as well as enzymes is paralyzed by certain poisons (HCN, H_2S).

4. Both classes of bodies are colloid substances and possess an enormous surface upon which their catalytic action depends.

According to NEILSON, ethyl butyrate,[4] salicin and amygdalin[5] are decomposed by platinum black as well as by enzymes.

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[1] Nernst and Brunner, *ibid.*, **47**, 52 and 56, 1904.

[2] Anorganische Fermente, Leipzig, 42, 1901.

[3] Zeitschr. f. physik. Chem., **31**, 288, 1899.

[4] Amer. Journ. of Physiol., **10**, 191, 1904.

[5] *Ibid.*, **15**, 148, 1906.

If those bodies which accelerate a reaction are to be considered as catalysts, then certainly the solvents must belong to the catalytes. Attention must be called to the enormous influence which the solvent can exert upon the velocity of a reaction under otherwise equal conditions. Thus MENSCHUTKIN [1] found for the reaction

$$(C_2H_5)_3.N + C_2H_5.I = (C_2H_5)_4.N.I.,$$

the following velocity in different solvents:

Hexane	0.00018
Heptane	0.000235
Xylene	0.00287
Benzene	0.00584
Ethyl alcohol	0.03660
Benzyl alcohol	0.13300

Recently BREDIG and FAJANS [2] have been able to show that an optically active solvent can help in the decomposition of optical antipodes to a varying extent. Of the optical antipodes of campho-carboxylic acid, the *d*-form is 17 per cent more quickly decomposed than the *l*-form, when they are dissolved in nicotine or when nicotine is present, dissolved with the catalyte, while in an optically indifferent solvent and without any nicotine the catalyte decomposes both forms with equal rapidity.

The reaction proceeds differently with or without catalyst, and the catalyst acts not only upon the velocity of the reaction. It is apparent that this does not conform with OSTWALD's definition of a catalyst (page 54).

IV. ENZYMES.

As the enzymes are active in very small amounts without undergoing any essential change, they have for a long time been included among the catalytic substances.

In regard to the terminology it must be remarked that those bodies upon which an enzyme acts is designated as the substrate, and often an enzyme is named after the substrate (amylase, protease, lipase); in other cases the kind of action determines the name (oxidase, reductase), and finally the name is founded upon the product produced by the action (alcoholase).

We have no general method for preparing the enzymes. In certain cases they are contained in secretions (gastric and pancreatic enzymes); in others they are prepared from the cells by crushing and pressing out the cell juice (zymase, organ enzymes), and finally, most enzymes can be extracted from the cells with water or glycerin, and as this last gives permanent solutions it has found great use as an extraction medium.

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[1] Zeitschr. f. physik. Chem., 6, 41, 1890.

[2] Ber. d. d. chem. Gesellsch., 41, 752, 1908.

In all these cases the enzymes are obtained strongly contaminated with other bodies. No enzyme has thus far been obtained in a perfectly pure form, and the chemical constitution as well as structure is therefore unknown. The enzymes probably belong to the colloids; if they themselves are not colloids, they occur at least with colloids, from which they cannot be separated. The enzymes are characterized by the fact that they are readily taken up by finely divided substances (inorganic precipitates, carbon, kaolin, infusorial earth and other colloids such as alumina, iron hydroxide, proteins [1]). This process may act selectively, as from a solution certain enzymes can be taken up and others not at all, or only to a slight extent (HEDIN,[2] MICHAELIS and EHRENREICH [3]).

All enzymes lose their specific action on sufficiently heating their aqueous solutions, and even at ordinary temperature the enzymes are gradually decomposed. MADSEN and WALBUM have followed this process at different temperatures and found that the decomposition of trypsin, pepsin and rennin at given temperatures proceeds monomolecularly, i.e., that the velocity of reaction at every moment is proportional to the concentration of the enzyme (page 54).[4] The readiness with which an enzyme is decomposed is nevertheless to a great extent dependent upon the presence of other bodies (page 61).

Certain enzymes are also sensitive to light. According to SCHMIDT-NIELSEN [5] chymosin is injured by light and indeed, by the ultra-violet rays. The experiments of JODLBAUER and TAPPEINER [6] with invertin have led to the same results; the visible rays also can in certain cases (peroxidase, hæmase) in the presence of oxygen or certain fluorescent substances exert an injurious action.[7]

Just as it is difficult to prepare an enzyme free from non-enzymotic contaminations, so also is it difficult to exclude the possibility that a so-called enzyme is not a mixture of several related enzymes. In fact the several enzymotic processes proceed step by step, and it is possible that the various steps are caused by different enzymes. This seems according to BUCHNER and collaborators [8] to be the case in alcoholic fermentation, in which the dextrose is probably first split into lactic

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[1] Dauwe, Hofmeister's Beiträge, **6**, 426, 1905.

[2] Bioch. Journ., **2**, 112, 1907.

[3] Bioch. Zeitschr., **10**, 283, 1908.

[4] Arrhenius, Immunochemie, Leipzig, 1907, 5°.

[5] Hofmeister's Beiträge, **5**, 355, 1904; **8**, 481 1906; Zeitschr. f. physiol. Chem., **58**, 233, 1908.

[6] Arch. f. klin. Med., **87**, 373, 1906.

[7] Bioch. Zeitschr., **8**, 61 and 84, 1908.

[8] Ber. d. d. chem. Gesellsch., **37**, 419, 1904; **38**, 620, 1905.

acid and this then into alcohol and carbon dioxide. In the same manner the decomposition of protein into amino acids, with proteoses, peptones, and polypeptides as intermediary products, may be the result of the activity of several enzymes which are active one after another or are parallel with one another in activity. Erepsin does not attack genuine proteins, but completes the decomposition which has been begun by other enzymes (pepsin, trypsin).

The enzymes are formed within the living cells. In certain cases the cells do not secrete the complete enzyme, but substances which are transformed first outside of the cells into active enzymes (*zymogens, proenzymes*). The best-studied example of a zymogen is trypsinogen, which is contained in the pancreatic juice and converted into an active enzyme by the enterokinase of the intestine. As this kinase is destroyed by heat it also seems to be an enzyme-like body. (See Chapter IX.) Trypsinogen can also be activated by lime salts. In certain other cases the presence of bodies which resist temperature and are dialyzable and therefore not enzymes, are necessary besides the real organic enzyme. Thus the presence of an acid is necessary for the action of pepsin. R. MAGNUS [1] has been able to separate by dialysis from a solution of liver-lipase a body which is necessary for the action upon amyl salicylate. Enzymes made inactive by dialysis can be activated again by the addition of boiled enzyme or the concentrated dialysate. HARDEN and YOUNG [2] on filtering yeast-press juice through earthenware filters impregnated with gelatin, have found different constituents of the zymase on the filter and in the filtrate. The true enzyme remains on the filter. This alone is inactive, but becomes active when the other part which has passed through the filter, and which is dialyzable and resistant to temperature, is added. According to BUCHNER and KLATTE [3] the constituent of the zymase which is resistant to temperature is destroyed by lipase. Certain of the just-mentioned substances which are resistant to heat, whose presence are necessary for the action of certain enzymes, are ordinarily called *co-enzymes*. As they are not to be classified with the enzymes, they are more correctly called *activators*, as suggested by EULER.[4] Their action is probably different in different cases, and differs also from the activating action of the kinases.

Laws of Action of the Enzymes. The enzyme reactions always take place in heterogeneous media, where on one hand the enzyme exists as colloid and on the other the substrate in many cases is a colloid (starch

[1] Zeitschr. f. physiol. Chem., 42, 149, 1904.

[2] Proc. Physiol. Soc., 32, 1904; Proc. Chem. Soc., 21, 189, 1905; Proc. Roy. Soc., 77 (ser. B), 405, 1906; *ibid.*, 78, 369, 1906.

[3] Bioch. Zeitschr., 8, 520, 1908.

[4] Zeitschr. f. physiol. Chem., 57, 92, 1908.

proteins). As above mentioned, the enzymotic decompositions are often complicated by their taking place over several intermediary steps to the final product. As indicated by several conditions, the enzymes also, before they act upon the substrate, combine therewith in some way or another. The fact that the action of an enzyme is dependent upon the stereometric construction (page 67) of the substrate speaks essentially for this view. The substrate also protects certain enzymes against destructive influences (heat, alkalies)[1]; casein is able to protect trypsin against the retarding influence of seralbumin (HEDIN [2]). Trypsin adsorbed by carbon is in part abstracted by proteins (HEDIN [3]). According to this only that part of the added enzyme which is combined with the substrate is active. We must now consider the following:

1. The velocity with which the enzyme combines with the substrate.
2. The result of the division, i.e., how much of the added enzyme is combined with the substrate.
3. The velocity of the chemical processes produced by the enzyme.

The velocity of the combination of the enzyme with the substrate (1) can at least in many cases be ignored in consideration of the time necessary for the chemical reaction. This applies to those cases where the chemical transformation in the presence of an excess of substrate at the beginning of the processes remains the same in each successive time interval. The quantity of enzyme combined with the substrate, does not, in these cases, increase with the time, which would be the case if the time necessary for the combination is not in comparison with that for the chemical reaction.

Equal decomposition for equal time at the beginning of the processes have been found for the following enzymes—invertase,[4] diastase,[5] trypsin with casein.[6]

The second question, as to the division of the enzyme between different bodies, we will discuss after we have spoken of the velocity of the real chemical reaction as well as the retardation of enzyme action.

In regard to question 3, it must be recalled that the following laws have been found for catalytic reactions:

1. When the quantity of catalyst remains constant, the reaction velocity for every moment is proportional to the concentration of the body decomposed, which is shown by the velocity coefficient in the same experiment being constant at different times.

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[1] O'Sullivan and Thompson, Journ. Chem. Soc., **57**, 926, 1890; Bayliss and Starling, Journ. of Physiol., **30**, 71, 1903; Hedin, *ibid.*, **30**, 173, 1903; **32**, 474, 1905; Taylor, Journ. of biol. Chem., **2**, 90, 1906.

[2] Journ. of Physiol., **32**, 390, 1905, also Zeitschr. f. physiol. Chem., **50**, 497, 1907.

[3] Bioch. Journ., **2**, 81, 1906.

[4] O'Sullivan and Thompson, Journ. Chem. Soc., **57**, 926, 1890; Ducleau, Traité de microbiologie II, 137; Brown, Trans. Chem. Soc., **81**, 373, 1902; Armstrong, Proc. Roy. Soc., **73**, 500, 1904.

[5] Brown and Gliddinning, Proc. Chem. Soc., **18**, 43, 1902.

[6] Hedin, Journ. of Physiol, **32**, 471, 1905.

2. The velocity coefficient or the reaction velocity with constant concentration of substrate is proportional to the quantity of catalyst.

The first law has been shown for certain enzymes in case an excess of enzyme is present, namely for invertin,[1] lactase[2] and trypsin.[3] It was found that the decomposition in a certain time was proportional to the substrate. In other cases the determination of the correctness of the law was accomplished with difficulty. A part of the enzyme may during an experiment be either destroyed or in other ways (combining with the product) be put out of action; then reverse reactions may take place (page 65) and finally in many cases our analytical methods are incapable of obtaining comparative results for different decompositions, as the reaction in many cases takes place step by step, or several reactions occur at the same time.[4] Only in a few cases with especially simple reactions have constant values been found for the velocity coefficient at the beginning, as long as the quantity of reaction product was small and the active quantity of enzyme remained unchanged. With the aid of the formula for a monomolecular reaction

$$k = \frac{1}{t} \log \frac{C}{C-x}$$

(page 54), constant values were obtained for the velocity coefficient k in the following cases:

1. Decomposition of H_2O_2 by catalase from blood (hæmase [5]).
2. Decomposition of H_2O_2 by catalase from Boletus scaber.[6]
3. Decomposition of ethyl butyrate by an enzyme from pig fat.[6]
4. Decomposition of amyl butyrate by an enzyme from the pancreas.[7]
5. Decomposition of triolein and triacetin by an enzyme from the castor oil seed.[8]
6. Decomposition of glycyl-glycine by erepsin.[9]

The reaction is in these cases monomolecular. Although probably most enzymotic cleavage processes proceed with an excess of water monomolecularly, still the proof of this is only exceptionally found. Indeed such an apparently simple reaction as the inversion of cane-

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[1] Brown, Proc. chem. Soc., **18**, 14, 1902.

[2] Armstrong, Proc. Roy. Soc., **73**, 500, 1904.

[3] Hedin, Journ. of Physiol., **32**, 475, 1905.

[4] Hedin, Zeitschr. f. physiol. Chem., **57**, 468, 1908.

[5] Senter, Zeitschr. f. physik. Chem., **44**, 257, 1903.

[6] Euler, Hofmeister's Beiträge, **7**, 1, 1906.

[7] Dietz, Zeitschr. f. physiol. Chem., **52**, 311, 1907.

[8] Taylor, Journ. of biol. Chem., **2**, 93, 1906; Nicloux, Compt. rend. soc. biol., **56**, 840, 1904.

[9] Euler, Zeitschr. f. physiol. Chem., **51**, 213, 1907.

sugar cannot be expressed by the above formula.[1] Recent investigations of HUDSON [2] show, however, that the inversion of cane-sugar by enzyme is a monomolecular reaction. The varying results of earlier investigators depend upon a disturbing action of the multirotation of the dextrose. These results have been confirmed by EULER.[3]

The second law for catalytic reactions which we have formulated that with constant quantities of substrate the reaction velocity is proportional to the quantity of enzyme, has been shown in certain cases where the substrate was in excess (practically constant quantity) namely with kephir lactase,[4] trypsin with casein as substrate.[5] In the just-mentioned monomolecular enzyme reactions the velocity coefficient in a few cases was found proportional to the quantity of enzyme (hæmase,[6] erepsin with glycyl-glycine as substrate,[7] pancreatic lipase [8]) and in others not (catalase from Boletus scaber,[9] lipase from pig fat)[9]. It has been shown for several enzymotic reactions that with the same substrate the same decomposition can be obtained if the time of action varies in inverse proportion to the added quantity of enzyme. If p is the quantity of enzyme and t the time of action, then the decomposition is the same in all tests where $p.t$ is the same figure. This rule has been found true for the following enzymes: invertin (O'SULLIVAN and THOMPSON under certain conditions[10]), pepsin (SJÖQVIST [11]), rennin (especially FULD [12]), peptone-splitting enzyme (VERNON[13]), fibrin ferment of snake poison (MARTIN[14]), trypsin (HEDIN[15]), pepsin, rennin, trypsin, pyocyaneus protease (MADSEN[16]). On the action of trypsin upon casein this law has been shown correct for different stages in the reaction.[17] This indicates that the progress of the entire reaction remains the same with different quantities of enzyme, only that the time for the same decomposition is inversely as the quantity

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[1] Henri, Zeitschr. f. physik. Chem., **39**, 194, 1901; Brown, Trans. Chem. Soc., **81**, 373, 1902.

[2] Journ. Amer. Chem. Soc., 1908.

[3] Pflanzen Chemie, Braunschweig, 1908, 80.

[4] Armstrong, Proc. Roy. Soc., **73**, 500, 1904.

[5] Hedin, Journ. of Physiol., **32**, 471, 1905.

[6] Senter, Zeitschr. f. physik. Chem., **44**, 257, 1903.

[7] Euler, Zeitschr. f. physiol. Chem., **51**, 213, 1907.

[8] Kastle and Loevenhart, Amer. Chem. Journ., **24**, 491, 1900.

[9] Euler, Hofmeister's Beiträge, **7**, 1, 1906.

[10] Trans. Chem. soc., **57**, 926, 1890.

[11] Skand. Arch. f. Physiol., **5**, 358, 1895.

[12] Hofmeister's Beiträge, **2**, 169, 1902.

[13] Journ. of Physiol., **30**, 334, 1903.

[14] *Ibid.*, **32**, 207, 1905.

[15] *Ibid.*, **32**, 468, 1905; **34**, 370, 1906.

[16] Arrhenius, Immunochemie, Leipzig, 1907, 46.

[17] Journ. of Physiol., **32**, 468, 1905; **34**, 370, 1906.

of enzyme. As clearly shown by HEDIN,[1] this indicates that the velocity coefficient is proportional to the quantity of enzyme which is called for by the second law. If we start with the above-mentioned assumption that only that enzyme is active which is combined, then it follows from the proportionality between the velocity coefficient and the quantity of enzyme, that always the same fraction of the enzyme is combined with the substrate, or that the division of the enzyme remains independent of the quantity. In regard to the division of the enzymes REICHEL and SPIRO [2] claim that rennin also during and after coagulation is divided according to a constant factor between the whey and curd.

In determining the quantity of enzyme the so-called SCHÜTZ's rule plays an important part. In its newest form this is, that the decomposition is proportional to the square root of the quantity of enzyme and the time, or decomposition $= k\sqrt{pt}$ where k is a constant, p the quantity of enzyme and t the time of the action. This was first shown by SCHÜTZ [3] for pepsin and indeed, in this form, decomposition $= k\sqrt{p}$ as the time (t) was constant. The form decomposition $= k\sqrt{pt}$ was given by SCHÜTZ, and HUPPERT.[4] According to PAWLOW this rule also applies to trypsin digestion.[5] SCHÜTZ's rule is good for a certain stage of digestion only and it indicates that the extent of the validity must be very dependent upon the method used for the determination of the decomposition as the different digestion products are determined by different methods. It must also be remarked that within the entire domain where SCHÜTZ's rule is applicable the same value for pt must correspond to the same decomposition, and necessarily the above-discussed enzyme-time rule must also be valid. SCHÜTZ's rule has also been proven for the action of gastric and pancreatic lipase.[6] According to ARRHENIUS [7] the validity of the rule can be explained by the assumption that the enzyme combines with the reaction products so that the active mass of enzyme changes in inverse proportion to the quantity of reaction products.

Reversibility. Many catalytic processes have been shown to be reversible, i.e., the same catalyst can influence the reaction in different directions according to the concentration of the substances present. Thus far we have only spoken of enzymotic *cleavages;* according to the above it is to be expected that also *synthetical* processes can be produced by enzymes.

The first example of such a reaction was given by CROFT-HILL.[8] He treated a 40 per cent dextrose solution with maltase at 30° C. for a

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[1] Zeitschr. f. physiol. Chem., **57**, 468, 1908.

[2] Hofmeister's Beiträge, **6**, 68, 1905.

[3] Zeitschr. f. physiol. Chem., **9**, 577, 1885.

[4] Pflüger's Arch., **80**, 470, 1900.

[5] Arbeit der Verdauungsdrüsen, Wiesbaden, 1898, 33.

[6] Stade, Hofmeister's Beiträge, **3**, 318, 1903; Engel, *ibid.*, see Fromme, *ibid.*, **7**, 77, 1906.

[7] Immunochemie, 1907, 43.

[8] Journ. of chem. Soc., **73**, 634, 1898.

very long time and concluded from the change in rotation and reduction power that some maltose was formed from the dextrose. EMMERLING[1] showed afterward that a synthesis of maltose did not occur, but rather an isomeric carbohydrate, isomaltose was formed, which is not split by maltase. According to ARMSTRONG[2] emulsin splits isomaltose, but not maltose, and therefore it can synthesize maltose from dextrose. A similar reaction had previously been shown by E. FISCHER and ARMSTRONG,[3] that kefir-lactase produced isolactose and not lactose from galactose and dextrose. According to CREMER[4] yeast-press juice has the power of forming glycogen from dextrose or levulose.

Of the enzymotic syntheses of protein-like substances nothing positive is known. Although it has been repeatedly claimed that the formation of plastein is a synthetical process, still the proofs for this are lacking. It is just possible that we are here dealing with the simultaneous precipitation of two or more digestion products.

A true reformation of a subtrate once split seems for the present not to have been proven for the carbohydrates and proteins. Such a synthesis of amygdalin is nevertheless known. Amygdalin is split by maltase into mandelic acid nitrileglucoside and dextrose. EMMERLING[5] has been able to reconstruct amygdalin from these products with the aid of maltase, and recently ROSENTHALER[6] reports that he has formed a so-called asymmetrical synthesis whereby from benzaldehyde and hydrocyanic acid under the influence of emulsin the *d*-form of the mandelic acid nitrile ($C_6H_5CH(OH).CN$) is formed. An undoubted synthesis of fat and other ester-like combinations of fatty acids is also known. KASTLE and LOEVENHART[7] have shown the formation of ethyl butyrate from ethyl alcohol and butyric acid under the influence of a pancreas enzyme. In an analogous manner HARRIOT[8] obtained monobutyrin from butyric acid and glycerin with blood serum. POTTEVIN[9] by means of a pancreas enzyme transformed oleic acid and glycerin into mono- and triolein as well as oleic acid esters with monatomic alcohols. The synthetical action of the pancreas has been closely studied by DIETZ.[10] The enzyme used by DIETZ was insoluble in water, and its action was

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[1] Ber. d. d. chem. Gesellsch., **34**, 600 and 2207, 1901.
[2] Proc. Roy. Soc. (ser. B), **76**, 592, 1905.
[3] Ber. d. d. chem. Gesellsch., **35**, 3151, 1902.
[4] *Ibid.*, **32**, 2062, 1899.
[5] Ber. d. d. chem. Gesellsch., **34**, 3810, 1901.
[6] Bioch. Zeitschr., **14**, 238, 1908.
[7] Amer. chem. Journ., **24**, 491, 1900.
[8] Compt. rend., **132**, 212, 1901.
[9] *Ibid.*, **136**, 1152, 1903; **138**, 378, 1903; Ann. Inst. Past., **20**, 901, 1906.
[10] Zeitschr. f. physiol., Chem., **52**, 279, 1907.

tested with *i*-amyl alcohol and *n*-butyric acid or the corresponding ester. It was shown that the reaction took place in the insoluble phase (enzyme). From the formula alcohol + acid $\rightleftarrows$ ester + water, it follows that when the molecular concentrations of alcohol, acid, ester and water are designated C_A, C_S, C_E, C_W, the reaction velocity of the ester formation for a homogeneous system is $\frac{dx}{dt} = k_1.C_A.C_S - k_2.C_E.C_W$ (see page 53), which equation can be simplified to $\frac{dx}{dt} = k_1.C_S - k_2.C_E$ as the alcohol and water were in excess and their concentration considered as constant and included in the constants k_1 and k_2. At equilibrium we have $k_1C_S = k_2C_E$ or $\frac{k_1}{k_2} = \frac{C_E}{C_S} = K$ (page 53). It follows that the same equilibrium is attained irrespective of whether we start with alcohol + acid, or ester + H_2O. The equilibrium is also independent of the antecedents as well as the quantity of enzyme.

On comparing the equilibrium constants (K) which are obtained with different quantities of ester or acid, it is shown that in the above equation $\sqrt{C_E}$ must be introduced instead of C_E in order to obtain constant values for K. In the saponification of the ester the reaction velocity is proportional to $\sqrt{C_E}$, and not C_E. According to DIETZ this is due to the fact that the system is a heterogeneous one, and that only that part of the ester which is absorbed by the solid phase (enzyme) takes part in the reaction. The velocity constant of the ester formation is shown to be proportional to the quantity of enzyme.

According to what was stated above (page 56), the equilibrium in a reversible reaction must be independent of the nature of the catalyst. This was not the case in DIETZ'S experiments. With picric acid as the catalyst another equilibrium was obtained than with the pancreas enzyme. With the acid as catalyst the equilibrium was moved toward the direction of the ester. While this action is not understood it may perhaps be explained by the fact that the system in one case was homogeneous and in the other case heterogeneous.

Similar observations that the enzymotic end-condition can be different from the stabile end-condition of the same system have previously been made by TAMMANN,[1] but in these cases generally so-called false equilibrium existed, which, for example, by the addition of more enzyme changed, so that the cleavage proceeds further. These false equilibria are generally caused by the enzyme being destroyed or put out of action in other ways. In DIETZ'S experiment the equilibrium was true in every regard, as this condition could be attained from both sides and was independent of the quantity of enzyme.

Specificity of Enzyme Action. It has been known for a long time that a great difference exists in regard to the action of enzymes in the sense that different enzymes act only upon certain classes of bodies

[1] Zeitschr. f. physiol. Chem., 16, 271, 1892.

(proteins, carbohydrates, fats). Then there also exist differences in the manner in which different enzymes of the same group influence different members of the same class (maltase, lactase, invertin). Finally, it is possible for one enzyme to attack one of two optical antipodes and the other not at all, or only to a slight degree. That optical antipodes are burnt with unequal facility in the organism was shown by E. FISCHER, and that of the numerous aldohexoses only three, *d*-dextrose, *d*-mannose and *d*-galactose, and of the ketohexoses only one, *d*-levulose are fermentable; and then that the synthetically prepared stereoisomeric glucosides behave differently with the enzymes. Thus of two isomeric glucosides, one methyl-*d*-glucoside, the (α) was attacked by yeast and the other (β) only by emulsin, while the corresponding methyl-*l*-glucosides were not split by either of these enzymes. The corresponding glucoside obtained from galactose behaves in a similar manner.[1] FISCHER also found that amygdalin, which is split into benzaldehyde, hydrocyanic acid and dextrose by emulsin, is split into mandelic acid nitrile-glucoside and dextrose by yeast, and from these products the first can be further decomposed by emulsin into benzaldehyde, hydrocyanic acid and dextrose.[2] In connection with these observations FISCHER presents the theory that for the action of an enzyme a certain correspondence in stereometric structure of the enzyme and substrate must exist; the enzyme must fit the substrate somewhat like a key fitting a lock.

Then followed similar observations of DAKIN,[3] who found that racemic mandelic acid ester, on incomplete hydrolysis by liver press-juice, yielded a strongly dextrorotatory acid, while the ester remaining was levorotatory. The dextrorotatory ester was more quickly hydrolyzed than the levorotatory ester. Finally, we must mention the recent investigations of FISCHER and ABDERHALDEN[4] on the cleavage of polypeptides by pancreas-press juice. From abundant material they concluded that those polypeptides which consist entirely of the optical forms of amino-acids occurring in nature are hydrolyzed and the others not. If in a racemic form besides a polypeptide consisting of natural amino-acids, another occurs also then only the first is hydrolyzed. Besides this other factors are also of importance. Thus *l*-leucyl-glycine is not hydrolyzed, although both constituents occur in nature. The size of the molecule seems also to be of importance, as mono-, di- and triglycyl-glycine are not split, while tetraglycyl-glycine is.

Retardation of Enzyme Action. In the first place the products of enzymotic activity have a retarding action upon the enzymes. In

[1] Zeitschr. f. physiol. Chem., **26**, 60, 1898 (collection of Fischer's works).
[2] Ber. d. d. chem. Gesellsch., **28**, 1508, 1896.
[3] Journ. of Physiol., **30**, 253, 1903; **32**, 199, 1905.
[4] Zeitschr. f. physiol. Chem., **46**, 52, 1905; **51**, 264, 1907.

certain cases this retardation can be explained by a reverse reaction (synthesis) (page 59), in others no reverse reaction can be detected. That the inversion of cane-sugar is retarded by invert sugar has been claimed by many (HENRI,[1] A. J. BROWN,[2] BARENDRECHT,[3] ARMSTRONG [4]), and indeed BARENDRECHT claims that dextrose as well as levulose has a retarding action, and that galactose has an even stronger retarding action than the direct cleavage products of cane-sugar. H. E. and E. F. ARMSTRONG [5] found that invertin, maltase and lactase are retarded by just those varieties of sugar which are produced by their activity.

The retarding action of amino-acids upon the decomposition of glycyl-*l*-tyrosine by yeast-press juice has recently been studied by ABDERHALDEN and GIGON.[6] They found that cleavage of peptides is retarded by those optically active amino-acids which occur in the proteins. This result is remarkable in consideration of the observations of FISCHER and ABDERHALDEN that only those polypeptides were split by pancreatic juice which are composed of natural optically active amino-acids (page 67).

There also exist proteins which act retardingly upon the digestion of other proteins. The tryptic digestion of easily split protein bodies is retarded by the difficultly digested white of egg (DELEZENNE and POZERSKI,[7] VERNON,[8] GOMPEL and HENRI,[9] HEDIN.[10] The white of egg takes up a part of the enzyme and makes this partly inactive. At this time we must also mention the retarding action which the proteolytic primary cleavage products (proteoses, peptones) exert upon digestion. These products are further split; a part of the enzyme is combined with the products and in this way prevented from dissolving new protein (HEDIN [10]).

It has been known for a long time that blood-serum is able to retard various enzymotic processes. Certain enzymes have their action retarded even by normal serum. According to HAMMARSTEN and RÖDÉN [11] normal horse-serum retards the coagulation of milk, and the antitryptic action of serum has been shown by several investigators (HAHN,[12] CAMUS and

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[1] Zeitschr. f. physik. Chem., **39**, 194, 1901.
[2] Journ. Chem. Soc., **81**, 382, 1902.
[3] Zeitschr. f. physik. Chem., **49**, 456, 1904.
[4] Proc. Roy. Soc. (ser. B), **73**, 516, 1904.
[5] *Ibid.*, **79**, 360, 1907.
[6] Zeitschr. f. physiol. Chem., **53**, 251, 1907.
[7] Compt. rend. soc. biol., **55**, 935, 1903.
[8] Journ. of Physiol., **31**, 495, 1904.
[9] Compt. rend. soc. biol., **58**, 457, 1906.
[10] Zeitschr. f. physiol. Chem., **52**, 422, 1907.
[11] Upsala läkare forenigs förh., **22**, 546, 1887.
[12] Ber. klin. Wochenschr., **34**, 499, 1897.

GLEY,[1] LANDSTEINER[2]). The serum can attain retarding action toward other enzymes by repeatedly injecting the enzyme into an animal in the same manner as the anti-sera are obtained for bacterial toxines. In this way HILDEBRANDT[3] first obtained an anti-enzyme, namely for emulsin, and MORGENROTH[4] has produced in the same manner an anti-rennin in goats' serum. According to EHRLICH the natural and the artificial anti-bodies are identical, which is not the case in the experiments of MADSEN and WALBUM,[5] at least for the natural and artificially produced anti-rennin.

On studying the retardation of tryptic digestion, HEDIN[6] found that in different cases a different process occurs. The retarding action of normal beef serum is connected with the seralbumin (LANDSTEINER,[7] CATHCART[8]). In this case the trypsin, according to HEDIN, is in some way or other partly attached in an irreversible manner to the retarding substance (see page 73), because much more trypsin is neutralized when the mixture is made in the order seralbumin-trypsin-substrate, than when the substrate is added to the trypsin before the seralbumin. As above stated, egg albumin and the tryptic digestion products also act retardingly upon tryptic digestion. In this case it is immaterial in what order the bodies are mixed, and the trypsin is therefore taken up in a perfectly reversible manner (*enzyme deviation*). On treating seralbumin with 0.1–0.2-per cent acetic acid at 37° C. it loses the power of attaching trypsin; it has a less retarding action than before, and now indeed in the same manner as egg albumin.

HEDIN[9] has also found that bone-charcoal has the remarkable ability of adsorbing trypsin, and chiefly in an irreversible manner. From this it follows that charcoal can retard the action of trypsin in a very pronounced manner; and many analogies between the retarding action of native seralbumin and that of carbon can be detected.

Antigens and Anti-bodies. In connection with the retardation of enzyme action we can also call attention to other similar processes. Under the name *antigen* we include those substances which, when injected into animals, cause the formation of bodies in the organism with which they can in some way or another react. The bodies thus formed are

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[1] Compt. rend. soc. biol., **49**, 845, 1897.
[2] Centralbl. f. Bact., **27**, 357, 1900.
[3] Virchow's Arch., **131**, 33, 1893.
[4] Centralbl. f. Bakt. u. Parasitenk., **26**, 349, 1899.
[5] See Arrhenius Immunochemie, 180.
[6] Zeitschr. f. physiol. Chem., **52**, 412, 1907.
[7] Centralbl. f. Bakt., **27**, 357, 1900.
[8] Journ. of Physiol., **30**, 156, 1903.
[9] Bioch. Journ., **1**, 484, 1906; **2**, 81, 1907.

called *anti-bodies*. Generally these anti-bodies are specific in the sense that they only react with the corresponding antigen. The chemical constitution of the antigen as well as of the anti-body is not known; they belong perhaps to the colloids, or at least they occur associated with colloids.

To the antigens belong in the first place certain poisonous substances of animal or plant origin (toxins), for example, snake poisons, bacterial poisons, ricin (from the seeds of *Ricinus communis*), also enzymes as well as certain proteins without special action. The reaction with the anti-bodies (which are obtained in the blood serum of animals) manifests itself with the poisons by the suppression of the poisonous action, with the enzymes by retardation of the enzyme action, and with certain proteins by formation of a precipitate which contains the antigen as well as the anti-body. Anti-bodies of this last type are called *precipitins*.

If certain cells, for example, bacteria, blood-corpuscles, and spermatozoa are injected into animals, then anti-bodies are formed which have been called *immune bodies* (also amboceptors or sensibilizators). By themselves the immune bodies are inactive, but form with *complements*, substances occurring in normal serum, so-called *cytotoxins*, which destroy the kind of cells active in their formation. These cytotoxins are called *bacteriolysins, hæmolysins*, etc., according to the kind of cells used. The immune bodies are specific and also stable against heat; the complements can act together with different immune bodies and are very unstable, as they are generally destroyed by heating to 56° C. for one-half hour. Other anti-bodies, produced under the influence of injected cells, show their action by flocking together and agglutinating the cells set free in their formation. These anti-bodies are called *agglutinins*.

The longest known (due to the epoch-making investigations of v. Behring [1]) and best studied are those anti-bodies which are produced by toxins and which neutralize the action of the toxins upon the animal organism (antitoxins). According to the older view this takes place by some sort of an action of the anti-body upon the cells sensitive to the toxins. After it was shown that the toxins could also be neutralized *in vitro* by the anti-bodies, it is now generally accepted that the neutralization is brought about by some sort of a combination between the toxin and the anti-body. The views are very contradictory in regard to the nature of this combination and the manner in which it is formed.

The oldest theory, which has contributed much to our knowledge of these conditions, is that of P. Ehrlich, whom we must thank for the method for measuring the quantity of toxin by injection into an ani-

[1] Deutsch. med. Wochenschr., 1892; Zeitschr. f. Hygiene, **12**, 1892.

mal. The quantity of toxin which is just sufficient to kill a guinea-pig of given weight in a certain time is selected as the unit. According to the so-called *side-chain theory* of EHRLICH [1] the toxins firstly have a so-called haptophore group, by means of which the toxin can attach itself to a certain cell, and secondly, a so-called toxophore group, by which the toxin exerts its poisonous action. The formation of anti-body after the injection of the toxins EHRLICH explains by the fact that those cells which are attacked by the toxins are supplied with so-called *receptors*, which just fit the haptophore group of the toxins; the toxins are thus anchored on the questionable cells and can then begin their action by aid of the toxophore group. By the attachment of the receptors, the cells are induced to produce new receptors, and indeed, so many receptors are produced that they are thrown off and appear free in the blood plasma. The receptors circulating in the blood are the anti-bodies. As these are able to combine with the toxins they can protect against the toxin those cells which are supplied with the same receptor under whose influence they were found. The toxophore group of the toxins can gradually be destroyed on keeping. A toxin so changed can be continuously anchored to cell-receptors and in this way form anti-bodies, but cannot produce any poisonous action. A toxin without toxophore groups is called a *toxoid* by EHRLICH. It follows that the toxoids can combine with the anti-bodies.

According to EHRLICH, on the neutralization of a toxin a chemical combination takes place between the toxin and the anti-body, and so much of this combination is formed that either the toxin or the anti-body is completely consumed. Now the bacterial poisons are not simple bodies, but mixtures of several poisons of different toxicity and different avidity toward the anti-bodies. Generally the most poisonous is first neutralized, but it also occurs that a less poisonous or indeed a non-poisonous body is first combined with the anti-body (proto-toxoids) or that non-poisonous bodies are combined parallel with the true toxins (syntoxoids). The less poisonous or non-toxic bodies first combined after the binding of the true toxins are called *toxons* (also epitoxoids). According to the relative quantity and the avidity of the different constituents of the toxic solution can the addition of a certain quantity of anti-body produce entirely different results.

In regard to the immune bodies, EHRLICH believes that they combine with those kind of cells under whose influence they have been formed and also with the complements. They serve to fasten (amboceptors) the complement, which produces the real poisonous action, to the cells. The immune bodies correspond therefore to the haptophore groups of

[1] See Michaelis, Die Bindungsgesetze von Toxin und Antitoxin, Berlin, 1905.

the anti-toxins and the complements of the toxophores. According to BORDET the immune bodies act upon the cells in the way that the latter are sensitive toward the complements (sensibilizators).

ARRHENIUS and MADSEN oppose EHRLICH'S theory that the combination between toxin and anti-body is of a chemical nature, but claim that their formation does not proceed until one of the components has been used up. An equilibrium is established between the free toxin and the free anti-body on one side and the combination of the two on the other, which the law of mass action requires according to the formula:

$$C_{\text{toxin}} \cdot C_{\text{anti-body}} = K \cdot C^{N}_{\text{toxin+anti-body}} \qquad \text{(page 53).}$$

For tetanolysin (a substance obtained from tetanus cultures, which dissolves red-blood corpuscles) and its anti-body, as well as for diphtheria toxin and the corresponding anti-body, $n=2$ was found, i.e., in the combination of a molecule of toxin with a molecule anti-body two molecules toxin-antitoxin combination was formed.

The toxic action which a mixture of toxin and anti-body exerts depends upon the quantity of toxin which, according to the above formula, must always remain free.[1] According to this theory the toxin is a unit poison, as ARRHENIUS [2] recently admits with EHRLICH, that the poison is gradually transformed into a non-toxic or only slightly toxic substance which has the same ability to combine with antitoxin as the toxin itself.

EHRLICH'S theory, as well as that of ARRHENIUS-MADSEN, admits of a chemical combination between the antigen and the anti-body. According to EHRLICH besides this the substrate (or the cells sensitive to the antigen) combines with the antigen, which is not conformable with the theory of ARRHENIUS-MADSEN.

The combination toxin-anti-body is first gradually produced, and then it is taken up from all sides so that the toxin is fastened to the anti-body by a secondary process (exception, cobra poison). The combination toxin-antitoxin is not reversible in the ordinary sense. This is most easily shown by the fact that to a certain limit more toxin is neutralized according to the time allowed to elapse before the quantity of toxin remaining free is determined by injection into an animal or in other ways.[3] In certain cases it is possible to obtain the toxin again in an active form from the toxin-antitoxin combination, and indeed by treatment with very dilute hydrochloric acid (MORGENROTH [4]). On the

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[1] Zeitschr. f. physik. Chem., 44, 7, 1903.

[2] Immunochemie, Leipzig, 1907, 132.

[3] Martin and Cherry, Proc. Roy. Soc., 1898, 420.

[4] Berl. klin. Wochenschr., 1905, No. 5; Festschr. z. Eröffnung d. pathol. Instit. Berlin, 1906; Virchow's Arch., 190, 371, 1907.

contrary, HEDIN could not obtain free trypsin from trypsin neutralized with native seralbumin, by treatment with very dilute acetic acid, although the acid was shown by special experiments to be able to destroy the binding properties of the seralbumin.[1]

Recently a third manner of considering the toxin-antitoxin reaction has been presented which is based on the fact that the reaction takes place in a heterogeneous system. According to this the reaction is considered as an adsorption process, and in support of this assumption, several examples can be given where finely divided solids or colloid substances take up toxins or enzymes and indeed, in an irreversible manner (NERNST,[2] BILTZ,[3] LANDSTEINER [4]).

V. IONS AND SALT ACTION.

We have already mentioned various processes which depend upon the influence of ions. To these belong the precipitation of suspension colloids by electrolytes as well as different catalytic processes. That in the last case we are dealing with the action of ions is proven by the fact that the velocity coefficient is proportional to the concentration of a certain kind of ion. Nevertheless it has been shown, that the velocity coefficient in the inversion of cane-sugar, by acid, is only proportional to the *H* ions when dilute acids are used. With greater concentration disturbances occur which can be ascribed to the action of the negative ions of the acids. The catalytic processes can be influenced by salts in a similar manner (salt action).

The enzyme action has shown itself proportional to the quantity of enzyme in certain cases. EULER [5] has attempted to show a correspondence between ion-action and enzyme action by the assumption that the enzymes cause an increase in those ions, which could cause the reaction without the presence of the enzyme.

Many enzymotic processes are influenced by the presence of salts of the alkalies or alkaline earths. According to BIERRY, GIAJA and HENRI as well as PRETI [6] pancreatic juice dialyzed for a long time has no action upon starch, but becomes active again on adding NaCl or other salts. According to WOHLGEMUTH [7] the diastatic power of saliva is increased ten-fold by the addition of NaCl. The anions are the active part in these cases. The strong retarding action which NaFl exerts upon the enzy-

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[1] Bioch. Journ., **1**, 479, 1906; Zeitschr. f. physiol. Chem., **50**, 497, 1907.
[2] Zeitschr. f. Elektrochem., **10**, 379, 1904.
[3] Ber. d. d. Chem. Gesellsch., **37**, 3147, 1904; Beitr. z. exp. Therapie, **1**, 30, 1905.
[4] Zeitschr. f. Chem. u. Ind. d. Koll., **3**, 221, 1907; Bioch. Zeitschr., **15**, 33, 1908.
[5] Zeitschr. f. physik. Chem. **36**, 641 1901.
[6] Compt. rend. soc. biol., **60**, 479, 1906; **62**, 432, 1907; Bioch. Zeitschr., **4**, 1, 1907.
[7] Bioch. Zeitschr., **9**, 1, 1908.

motic cleavage of esters is also to be mentioned (LOEVENHART and PIERCE, AMBERG AND LOEVENHART [1]).

Other actions of salts are also ascribed to ion-action. To these belong the experiments of DRESSER,[2] according to which mercury salts, which are relatively strongly dissociated, have a poisonous action upon organic formations (yeast, frog heart), while potassium-mercury hyposulphite was nearly non-toxic. As the last-mentioned salt contains very few free *H* ions the poisonous action of the first salt is ascribed to the ions. PAUL and KRÖNIG [3] have arrived at similar results by investigating the poisonous action of mercury salts upon spores. They found that K_2Cy_4Hg, which hardly contains any *Hg* ions, is much less poisonous than an equivalent solution of $HgCy_2$. The same conditions were observed by MAILLARD [4] for copper salts.

In this connection it is interesting to mention the interesting investigations of J. LOEB [5] on the artificial fertilization of eggs of lower sea animals. LOEB found that it is possible to obtain larvæ from the unfertilized eggs of the sea-urchin, *Arbacia*, which never develop in normal sea-water. For this purpose it was only necessary to keep the eggs for two hours in sea-water whose osmotic pressure had been raised 40–50 per cent and then placing the eggs again in normal sea-water. It is immaterial how the osmotic pressure is raised, whether by the addition of NaCl, KCl, $MgCl_2$, urea, or sugar. It is evident that the bodies used are those which cause an effective osmotic pressure. LOEB ascribes their action to their ability to attract water. The development of the egg does not take place exactly like an egg fertilized with spermatozoa. LOEB obtained much better results by using the eggs of another sea-urchin, *Strongylocentrotus*, which he allows to lie first for ½–1 minute in sea-water slightly acidulated with a low fatty acid, for instance formic acid, and then replaced in normal sea-water. By this means chemical processes are introduced, which when the eggs remain in sea-water causes death in less than 24 hours, but if the eggs, 5–10 minutes after the membrane formation which takes place in sea-water, are kept for 20–45 minutes in hypertonic sea-water, and then again placed in normal sea-water, the development of active larvæ is the result. The presence of oxygen is necessary for the result of the experiment. LOEB has also obtained larvæ from other

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[1] Journ. of Biol. Chem., **2**, 397, 1907; **4**, 149, 1908.

[2] Arch. exp. Pathol. u. Pharm., **32**, 456, 1893.

[3] Zeitschr. f. physik. Chem., **31**, 411, 1896.

[4] Compt. rend. soc. biol., **50**, 1210, 1898.

[5] Loeb, Vorlesungen über die Dynamik der Lebenserscheinungen, 1906, 243; Pflüger's Arch., **118**, 572, 1907; Ueber den chemischen Charakter des Befruchtungsvorganges, Leipzig, 1908.

animal forms by the aid of methods adhering more or less closely to the above.

Certain ions or salts have an antagonistic action upon the animal organs. NaCl has in general a beneficial influence upon animal tissues. Nevertheless, pure NaCl solutions can have a poisonous action, for example upon frog muscles. In such cases the toxic action is arrested by the addition of small quantities of other salts, namely K and Ca salts. Upon this depends the use, in experiments with animal organs, of the so-called RINGER-LOCKE [1] solution, which contains in a liter about the following substances: 6.5–9.5 grams NaCl + 0.2 grams KCl + 0.2–0.3 gram $CaCl_2$, and also 0.1 gram $NaHCO_3$. The experiments of LOEB with the fertilized eggs of the *Fundulus heteroclitus* are very interesting. These eggs develop just as well in a remarkable manner in water free from salt as in sea-water, and are completely insensible to osmotic influence. But if the fertilized eggs are placed in a NaCl solution of the same osmotic pressure as the sea-water they die; the toxicity of the NaCl solution can be arrested by small quantities of nearly any salt with polyvalent cations. Not only the salts of the alkaline earths, but also those of the heavy metals can neutralize the toxicity of the NaCl in proper concentration.[2] WO. OSTWALD has carried on similar experiments with *Gammarus pulex*.[3]

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[1] Journ. of Physiol., 18, 318, 1895.
[2] Pflüger's Arch., 88, 68, 1901.
[3] *Ibid.*, 106, 568, 1905.

CHAPTER III.

THE PROTEIN SUBSTANCES.

THE chief mass of the organic constituents of animal tissues consists of amorphous nitrogenized, very complex bodies of high molecular weight. These bodies, which are either proteins in a special sense or bodies nearly related thereto, take first rank among the organic constituents of the animal body on account of their great abundance. For this reason they are classed together in a special group which has received the name *protein group* (from πρωτεύο, I am the first, or take the first place). The bodies belonging to these several groups are called *protein substances,* although in a few cases the protein bodies in a special sense are designated by the same name.

The several *protein substances* [1] contain *carbon, hydrogen, nitrogen,* and *oxygen.* The majority contain also *sulphur,* a few *phosphorus,* and a few also *iron. Copper, chlorine, iodine,* and *bromine* have been found in some few cases. On heating the protein substances they gradually decompose, producing a strong odor of burnt horn or wool. At the same time they produce inflammable gases, water, carbon dioxide, ammonia, and nitrogenized bases, besides many other substances, and leave a large quantity of carbon. On hydrolytic cleavage they all yield, besides nitrogenous basic substances, especially large amounts of α-monamino-acids of different kinds.

The nitrogen occurs in the protein bodies in various forms, and this is also revealed in the division of the nitrogen among the cleavage products. On boiling with dilute mineral acids we obtain (1) so-called amide nitrogen, which is readily split off as ammonia; (2) a guanidine residue which is combined with diaminovalerianic acid as arginine, and which has also been called the urea-forming group; (3) basic nitrogen or diamino-acid nitrogen, which is precipitated by phosphotungstic acid as basic products (to which also the guanidine residue of arginine

[1] See "Eiweisskörper," Ladenburg's Handwörterbuch der Chemie, 3, 534–589, which gives a complete summary of the literature of protein substances up to 1885. The more recent literature up to the year 1903 may be found in O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1904. See aslo Mann, Chemistry of the Proteids, London, 1906, and Oppenheimer's Handbuch der Biochem. der Menschen und der Tiere, 1908.

belongs); (4) monamino-acid nitrogen; and (5) the nitrogen in variable amounts which appears as humus-like melanoidins, which seem to be of only secondary formation as products of elaboration.

The quantitative division of the total nitrogen between the above five groups is different in the various protein substances, and moreover cannot be given with certainty, because of the above-mentioned melanoidin formation and the errors in the methods used.[1] The following gives at least an approximate idea of this division.[2] The loosely combined so-called amide nitrogen seems to be entirely absent in the protamines. In the gelatins we find 1–2 per cent, and 5–10 per cent in other animal protein substances,[3] in the plant gluten-proteids, 13–20 per cent of the total nitrogen is amide nitrogen. The guanidine nitrogen may amount in the protamines to 22–44 per cent of the total nitrogen, in the histones to 12–13 per cent, in the gelatins about 8 per cent, and in the other protein bodies about 2–5 per cent. As basic nitrogen precipitable by phosphotungstic acid (including the guanidine residue) we find 35–88 per cent in the protamines, 35–42.5 per cent in the histones, 15–30 per cent in the other animal protein substances, 5–14 per cent in zein and the gluten proteid, and up to 37 per cent in the plant globulins. The chief quantity of the nitrogen, 55–76 per cent, occurs, with the exception of the protamines, as the monamino-acid groups. The results for the melanoidin nitrogen vary so considerably that they will not be mentioned. Osborne, Leavenworth and Brautlecht[4] have recently published the results of their investigations on the different forms of binding of the nitrogen in the plant-proteins.

From the above results it follows that the nitrogen of most protein bodies exists in such combination that the chief quantity appears in the cleavage products as amino-compounds on hydrolytic cleavage by acids. By the action of nitrous acid upon proteins only a very small part, 1–2 per cent, of the nitrogen is evolved,[5] which seems to indicate that NH_2 groups exist only to a slight extent in protein substances. This assump-

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[1] See the work of Hausmann, Zeitschr. f. physiol. Chem., **27** and **29**; Henderson, *ibid.*, **27**; Kossel and Kutscher, *ibid.*, **30**; Kutscher, *ibid.*, **31**, **38**; Hart, *ibid.*, **33**; Gümbel, Hofmeister's Beiträge, **5**; Rothera, *ibid.*

[2] See the works given in footnote 1 and Blum, Zeitschr. f. physiol. Chem., **30**; Kossel, Ber. d. d. chem. Gesellsch., **34**, 3214; Hofmeister, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 759, which also contains the literature; Osborne and Harris, Journ. Amer. Chem. Soc., **25**; and Gümbel, l.c.

[3] Skraup and v. Hardt-Stremayr, Monatsh. f. Chem., **29**, found lower results than other investigators and they found also that about two-thirds of the amide nitrogen was readily split off and one-third slowly.

[4] Amer. Journ. of Physiol., 23.

[5] See C. Paal, Ber. d. d. chem. Gesellsch., **29**; H. Schiff, *ibid.*, 1354; O. Loew, Chemiker Zeitung, 1896; and O. Nasse, Pflüger's Arch., **6**.

tion does not have sufficient foundation, for, according to LEVITES,[1] the quantity of amide nitrogen is not diminished by the action of nitrous acid upon the protein substances. SKRAUP and co-workers have in part obtained similar results, and the deamidized proteins, the *desamidoprotein* at least in certain cases, do not strikingly differ in elementary composition from the original mother-substance. The so-called deamidation of proteins seems to be a rather complicated process whose nature is not well understood,[2] and therefore no positive conclusions can be drawn.

If by the action of HNO_2 only acid amide groups are attacked, then we should expect that the corresponding deamidized protein would yield on hydrolysis the same amino acids as the mother substance. If, on the contrary, the nitrous acid reacts also with NH_2 groups of the amino acids with an exchange of OH for NH_2, then it is apparent that on the hydrolysis of the diamino protein we would find other cleavage products, namely, oxyacids instead of amino acids, and either oxyacids or oxyamino acids instead of diamino acids. By the absence of certain cleavage products and the occurrence of others, we can with probability determine which of the cleavage products were so combined that one or the other NH_2 groups was free. From this standpoint SKRAUP and his co-workers have studied the hydrolytic cleavage products of the diamino proteins, and have found that these products, with the exception of lysin and in certain cases, a part of the arginine, were the same qualitatively and quantitatively as from the mother substance. Nearly all amino acids occur, it seems, in the investigated proteins in a form of binding when the amino groups are not free.

The chief part of the nitrogen in the proteins exists as an imide-like binding of the amino-acids, but other forms of bondage may occur at the same time. This will be developed in the following pages.

The sulphur occurs in the different proteins in very different amounts. Certain of them, such as the protamines and apparently also certain bacterial proteids,[3] are free from sulphur; some, such as gelatin and elastin, are very poor in sulphur, while others, especially horn substances, are relatively rich in sulphur. On hydrolytic cleavage with mineral acids, the sulphur of the protein substances is regularly, at least in part, split off as cystine (K. MÖRNER) or, with bodies poorer in sulphur, as cystein (EMBDEN), but this, according to MÖRNER and PATTEN, is a secondary formation. From certain protein substances α-thiolactic acid (SUTER, FRIEDMANN, FRÄNKEL), which MÖRNER claims is also produced secondarily mercaptans and sulphuretted hydrogen (SIEBER and

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[1] Levites, Zeitschr. f. physiol. Chem., **43**.

[2] On the deamidation of proteins and their cleavage products see footnote 5, page 77; Treves and Salomone, Biochem. Zeitschr., **7**; Skraup, Monatsh. f. Chem., **27** and **28**, with Hoernes, *ibid.*, **27**, with Kaas, Annal. d. Chem. u. Pharm., **351**; Lampel, Monatsh. f. Chem., **28**; Traxl, *ibid.*, **29**.

[3] See Nencki and Schaffer, Journ. f. prakt. Chem. (N. F.), **20**, and M. Nencki, Ber. d. d. chem. Gesellsch., **17**.

SCHOUBENKO, RUBNER), and a body having the odor of ethyl sulphide (DRECHSEL) have been obtained.[1]

A part of the sulphur separates as potassium or sodium sulphide on boiling with caustic potash or soda, and may be detected by lead acetate and quantitatively determined (FLEITMANN, DANILEWSKY, KRÜGER, FR. SCHULZ, OSBORNE, K. MÖRNER[2]). What remains can be detected only after fusing with potassium nitrate and sodium carbonate and testing for sulphates. The ratio between the sulphur split off by alkali and that not split off is different in various proteins. No conclusions can be drawn from this in regard the number of forms of combination which the sulphur has in the protein molecule. As shown by K. MÖRNER, only about three-fourths of the sulphur in cystine can be split off by alkali, and the same is true for the cystine-yielding complex of the protein substances. If the quantity of lead-blackening sulphur in a protein body be multiplied by $\frac{4}{3}$, we obtain the quantity corresponding to the cystine sulphur in the body. By such calculation MÖRNER found in certain bodies, such as horn substance, seralbumin and serglobulin, that the quantity of cystine sulphur and total sulphur were identical, and therefore we have no reason for considering the sulphur in these bodies as existing in more than one form of combination. In other proteins, such as fibrinogen and ovalbumin, on the contrary, only one-half or one-third of the sulphur appeared as cystine sulphur.

According to RAIKOW[3] keratin-like proteins split off sulphur dioxide on treatment with phosphoric acid at ordinary temperatures; hence it follows that a part of the sulphur in the proteins, especially in the keratins, exists in direct combination with oxygen and probably combined as in the sulphites.

The constitution of the protein bodies is still unknown, although the great advances made in the last few years have brought us essentially closer to the elucidation of the question. In studying the constitution of the protein bodies they have been broken up in various ways into simpler portions, and the methods used for this purpose have been of different kinds. In such decompositions, for which proteins have been used that can be prepared in the crystalline form, first large atomic complexes—proteoses and peptones—are obtained which still have

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[1] K. Mörner, Zeitschr. f. physiol. Chem., **28**, **34**, and **42**; Patten, *ibid.*, **39**; Embden, *ibid.*, **32**; Suter, *ibid.*, **20**; Friedmann, Hofmeister's Beiträge, **3**; Sieber and Schoubenko, Archiv d. sciences biol. de St. Pétersbourg, **1**; Rubner, Arch. f. Hygiene, **19**; Drechsel, Centralbl. f. Physiol., **10**, 529; Fränkel, Sitzungsber. d. Wien. Akad., **112**, II *b*, 1903.

[2] Fleitmann, Annal. der Chem. und Pharm., **66**; Danilewsky, Zeitschr. f. physiol. Chem., **7**; Krüger's, Pflüger's Archiv, **34**; F. Schulz, Zeitschr. f. physiol. Chem., **25**; Osborne, Connecticut Agric. Expt. Station Report 1900; Mörner, l. c.

[3] See Biochem. Centralbl., **4**, p. 353.

protein characteristics, and these then suffer further decomposition until finally we obtain simpler, generally crystalline, or at least well-characterized, end products.

On heating protein with barium hydroxide and water in sealed tubes to 150-250° C., SCHUTZENBERGER [1] obtained a mixture of products among which were ammonia, carbon dioxide, oxalic acid, acetic acid, and, as chief product, a mixture of amino-acids. The conclusion he drew from this experiment, that the protein is a complex ureide or oxamide, cannot be considered for several reasons.[2]

On fusing proteins with caustic alkali we obtain ammonia, methyl mercaptan, and other volatile products; also leucine, from which then volatile fatty acids, such as acetic acid, valeric acid, and also butyric acid are obtained, and also tyrosine, from which latter phenol, indol, and skatol are produced.

As to the products obtained by hydrolytic cleavage with mineral acids, we have a number of investigations by various experimenters, especially HLASIWETZ and HABERMANN, RITTHAUSEN and KREUSLER, E. SCHULZE and his collaborators, DRECHSEL, SIEGFRIED, R. COHN, KOSSEL and his pupils, K. MÖRNER, ABDERHALDEN and co-workers, OSBORNE and CLAPP, SKRAUP, and recently E. FISCHER and his collaborators.[3] The chief products thus obtained are monamino acids, such as glycocoll, alanine, aminovaleric acid, leucine, phenylaminopropionic acid, aspartic and glutamic acids, cysteine and its sulphide cystine; the so-called hexone bases, lysine, arginine, and histidine, of which the first two are diamino acids; tyrosine, oxymonamino acids, such as serine, oxyaminosuccinic acid, and oxyaminosuberic acid; oxydiamino acids, such as oxydiaminosuberic acid, oxydiaminosebacic acid, diaminotrioxydodecanoic acid, caseanic and caseinic acids; α-pyrrolidine and oxypyrrolidine carboxylic acids; tryptophane (indolaminopropionic acid); sulphuretted hydrogen, ethyl sulphide, leucinimide, ammonia, and melanoidins,[4] which latter seem to be secondary condensation products.

The proteins can be split into a large number of bodies by the proteolytic enzymes, and these will be presented later. In the first place proteoses and peptones are produced, also an abundance of monamino-acids of different kinds, hexone bases, tryptophane, and finally oxyphenylethylamine, diamines, and a little ammonia and other substances.

A great many substances are produced in the putrefaction of proteins. First the same bodies as are formed in the decomposition by

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[1] Annal. de chim. et phys. (5), **16**, and Bull. Soc. chim., **23** and **24**.

[2] See Habermann and Ehrenfeld, Zeitschr. f. physiol. Chem., **30**.

[3] In regard to the literature see O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1904, and F. Hofmeister, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759, 1902; E. Fischer, Untersuchungen über Animosäuren, Polypeptide und Proteine (1899–1906), Berlin, 1906; also Mann, Chemistry of the Proteids, London, 1906. See also special references.

[4] See Samuely, Hofmeister's Beiträge, **2**.

means of proteolytic enzymes are produced, and then a further decomposition occurs with the formation of a large number of bodies belonging in part to the aliphatic and in part to the aromatic and heterocyclic series. Of the first series we have ammonium salts of volatile fatty acids, such as caproic, valeric, and butyric acids, also succinic acid, carbon dioxide, methane, hydrogen, sulphuretted hydrogen, methyl mercaptan, and others. The ptomaines also belong to these products, and are probably in part formed by very different chemical processes, or even syntheses.

E. Salkowski divides the putrefactive products of the aromatic and heterocyclic series into three groups: (*a*) the phenol group, to which tyrosine, the aromatic oxyacids, phenol, and cresol belong; (*b*) the phenyl group, including phenylacetic acid and phenylpropionic acid; and lastly (*c*) the indol group, which includes indol, skatol, indol propionic acid and indol acetic acid. These various products are formed during putrefaction with access of air. Nencki and Bovet[1] obtained only *p*-oxyphenylpropionic acid, phenylpropionic acid, and skatolacetic acid on the putrefaction of proteins by anaerobic schizomycetes in the absence of oxygen. These three acids are produced by the action of nascent hydrogen on the corresponding amino-acids, namely, tyrosine, phenylaminopropionic acid, and skatolaminoacetic acid (indolaminopropionic acid), and according to Nencki these three last-mentioned amino-acids exist preformed in the protein molecule.

By the moderate action of chlorine, bromine, or iodine upon proteins, these halogens enter into more or less firm combination with the molecule (Loew, Blum, Blum and Vaubel, Liebrecht, Hopkins and Brook, Hofmeister, Kurajeff, and others), and according to the method of procedure we can prepare derivatives having different but constant amounts of halogens (Hopkins and Pinkus). The proteins are so changed that they do not split off sulphur on treatment with alkali, nor do they respond to Millon's reaction, nor do they yield tyrosine as a cleavage product. According to Schmidt, oxidations and cleavages may take place, as secondary processes, but more probably a substitution of hydrogen by halogen occurs in the aromatic nucleus of tyrosine or phenylaminopropionic acid and perhaps also in the indol nucleus of tryptophane and the imidazol nucleus of histidine.[2]

[1] Salkowski, Zeitschr. f. physiol. Chem., **12**, 215, and **27**, 302; Nencki and Bovet, Monatshefte f. Chem., **10**.

[2] Loew, Journ. f. prakt. Chem. (N. F.), **31**; Blum, Münch. med. Wochenschr., 1896; Blum and Vaubel, Journ. f. prakt. Chem. (N. F.), **57**; Liebrecht, Ber. d. deutsch. chem. Gesellsch., **30**; Hopkins and Brook, Journ. of Physiol., **22**; Hopkins and Pinkus, Ber. d. deutsch. chem. Gesellsch., **31**; Hofmeister, Zeitschr. f. physiol. Chem., **24**; Kurajeff, *ibid.*, **26**; Oswald, Hofmeister's Beiträge, **3**; C. H. L. Schmidt, Zeitschr. f. physiol. Chem., **35**, **36**, **37**; Neuberg, Biochem. Zeitschr., **6**: Pauly and Gundermann, Ber. d. d. chem. Gesellsch., **41**.

By the oxidation of protein by means of potassium permanganate, MALY obtained an acid, *oxyprotosulphonic acid*, C 51.21, H 6.89, N 14.59 S 1.77, O 25.54 per cent, which is not a cleavage product, but an oxidation product in which the group SH is changed into $SO_2.OH$. This acid does not give the proper color reaction with MILLON'S reagent, yields no tyrosine or indol, but gives benzene on fusing with alkali. On continued oxidation MALY obtained another acid, *peroxyproteic acid*, which gives the biuret reaction, but is not precipitated by most protein precipitants. The *oxyprotein* obtained by SCHULZ on the oxidation of protein by hydrogen peroxide is closely related to oxyprotosulphonic acid in composition and general characteristics, but contains lead-blackening sulphur and gives MILLON'S reaction. The oxyprotein is claimed to be a pure oxidation product, while in the production of oxyprotosulphonic acid SCHULZ claims that a cleavage takes place. According to the recent investigations of v. FÜRTH[1] there exist at least three different peroxyproteic acids (from casein) which differ from each other by a different division of the nitrogen in the molecule. On treatment with baryta-water we find that they split off basic complexes and oxalic-acid groups, and new bodies, the *desaminoproteic acids*, which give the biuret reaction, are produced. These acids, which on hydrolysis give benzoic acid but no diamino-acids, may be further oxidized, which is not true of the peroxyproteic acids, and yield a new group of acids, the *kyroproteic acids*, which give the biuret reaction, hold about one-half of their nitrogen (11.08 per cent total nitrogen) in acid-amide-like combination, but yield neither basic products nor benzoic acid.

On the oxidation of gelatin or protein with permanganate we also obtain oxaminic acid, oxamide, oxalic acid, oxaluric-acid amide, succinic acid, several volatile fatty acids, and guanidine, which was first shown by LOSSEN as an oxidation product (KUTSCHER, ZICKGRAF, SEEMANN, KUTSCHER and SCHENCK).[2]

On the oxidation of gelatin by ferrous sulphate and hydrogen peroxide BLUMENTHAL and NEUBERG have obtained acetone as a product, and ORGLER the same from ovalbumin. By the action of ozone upon casein, HARRIES and LANGHELD [3] found neither phenyl alanine nor tyrosine among the cleavage products, which fact they explain by the destructive action of the ozone upon the aromatic nucleus. Besides this, reducing bodies which are not carbohydrates, but which react with phenylhydrazine, are produced. JOLLES [4] claims to have obtained large quantities of urea in the oxidation of various proteins by potassium permanganate in acid solution, but this has been disputed by other investigators and the above statements in regard to the oxidation products of proteins are of little interest.

Nitric acid gives various nitro-products such as trinitroalbumin, oxynitroalbumin, xanthoprotein and others. A melanoidin substance, *xanthomelanin*, has been obtained by v. FÜRTH.[5] HABERMANN and EHRENFELD [6] also obtained oxyglutaric acid among other products. By the action of bromine under strong pressure a number of products have been obtained: bromanil and tribromacetic acid, bromoform, leucinimide, leucine, oxalic acid, tribromamino-

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[1] Maly, Sitzungsber. d. k. Akad. d. Wissensch., Wien, **91** and **97**. Also Monatshefte f. Chem., **6** and **9**. See also Bondzynski and Zoja, Zeitschr. f. physiol. Chem., **19**; Bernert, *ibid.*, **26**; Schulz, *ibid.*, **29**; v. Fürth, Hofmeister's Beiträge, **6**.

[2] Lossen, Annal. d. Chem. u. Pharm., **201**; Kutscher, Zeitschr. f. physiol. Chem., **32**; Zickgraf, *ibid.*, **41**; Seemann, *ibid.*, **44**; Kutscher and Schenck, Ber. d. d. chem. Gesellsch., **37** and **38**.

[3] Blumenthal and Neuberg, Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, **1**; Harries and Langheld, Zeitschr. f. physiol. Chem., **51**.

[4] Zeitschr. f. physiol. Chem., **32** and **38**.

[5] See Maly's Jahresber., **30**, 24.

[6] Zeitschr. f. physiol. Chem., **35**.

benzoic acid, and other bodies. With aqua regia, fumaric acid, oxalic acid, chlorazol, and other bodies are obtained. The investigations of HABERMANN and EHRENFELD and PANZER [1] upon the action of chlorine upon proteins and closely related products are important.

By the dry distillation of proteins we obtain a large number of decomposition products having a disagreeable burnt odor, and a porous glistening mass of carbon containing nitrogen is left as a residue. The products of distillation are partly an alkaline liquid which contains ammonium carbonate and acetate, ammonium sulphide, ammonium cyanide, an inflammable oil, and other bodies, and a brown oil which contains hydrocarbons, nitrogenized bases belonging to the aniline and pyridine series, and a number of unknown substances.

The occurrence of protein substances which contain a carbohydrate group has been known for a long time. The nature of this carbohydrate, which can be split off by acid and which may amount to as much as 35 per cent, has been explained chiefly by the investigations of FRIEDRICH MÜLLER [2] and his students. They have shown that it is always an amino-sugar, and generally glucosamine. That so-called true proteins also yield a carbohydrate on hydrolytic cleavage was first shown by PAVY, using ovalbumin. The continued investigations of FR. MÜLLER, WEYDEMANN, SEEMANN, FRÄNKEL, HOFMEISTER, and LANGSTEIN [3] have demonstrated that in these cases the carbohydrate is also glucosamine. A carbohydrate complex, although sometimes only to a very slight amount, has also been detected in other proteins, ovoglobulin, serglobulin, seralbumin, peaglobulin, albumin of the gramineæ, yolk-proteid, and fibrin. In other proteins, on the contrary, such as edestin (of the hemp-seed) and casein, myosin, pure fibrinogen, and ovovitellin, carbohydrates have been sought for with negative results. All proteins hence do not contain a carbohydrate group, and future investigators must therefore decide whether the carbohydrate groups belong positively to the protein complex or whether they are united with the protein only as impurities. Several observations [4] show that in working with crystalline proteins a contamination with other protein substances is unfortunately not excluded, and this must not be lost sight of, especially as the quantity of carbohydrates obtained is often very small. In the present state of our knowledge we are not warranted in considering the carbohydrate groups as belonging to the carbon nucleus produced on the destruction of the real protein complex.

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[1] Habermann and Ehrenfeld, Zeitschr. f. physiol. Chem., **32**; Panzer, *ibid.*, **33** and **34**.

[2] Müller, Sitzungsber. d. Ges. d. Natuw. zu Marburg, 1896 and 1898, and Zeitschr. f. Biologie, **42**.

[3] In regard to the literature on this subject see the work of Fr. Müller, Zeitschr. f. Biologie, **42**, and Langstein, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 63, Zeitschr. f. physiol. Chem., **41**, and Hofmeister's Beiträge, **6**. See also Abderhalden, Bergell, and Dörpinghaus, Zeitschr. f. physiol. Chem., **41**.

[4] See Wichmann, Zeitschr. f. physiol. Chem., **27**, and N. Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903, 51.

The previously mentioned methods used in studying the structure of the protein substances are not of the same value, but they in part substantiate each other. Of these we must mention the hydrolysis by means of boiling dilute mineral acids, or by proteolytic enzymes, as the best methods for obtaining the carbon nuclei in the protein molecule. The most important of the carbon nuclei obtained are as follows:

I. The Nuclei belonging to the Aliphatic Series.

A. *Sulphur free, but containing nitrogen:* 1. A *guanidine residue* (combined with ornithine as arginine). 2. *Monobasic monamino-acids:* Glycocoll, alanine, valine, leucine, and isoleucine. 3. *Bibasic monamino-acids:* Aspartic acid and glutamic acid. 4. *Oxymonamino-acids:* serine oxyaminosuccinic acid and oxyaminosuberic acid. 5. *Monobasic diamino-acids:* Diaminoacetic acid, ornithine (from arginine) and lysine. 6. *Oxydiamino-acids:* Oxydiaminosuberic acid, oxydiaminosebacic acid, diaminotrioxydodecanoic acid, caseanic and caseinic acids.

B. *Sulphurized:* Cysteine and its sulphide cystine, thiolactic acid, mercaptans, and ethyl sulphide.

II. The Nuclei belonging to the Carbocyclic Series.

Phenylaminopropionic acid and tyrosine.

III. The Nuclei belonging to the Heterocyclic Series.

Proline, oxyproline, tryptophane and histidine.

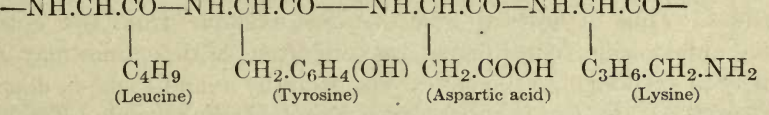
In regard to these carbon nuclei it must be remarked that they are not all found in every protein body thus far investigated, and also that one and the same cleavage product, such, for example, as glycocoll, leucine, tyrosine, etc., is obtained in very variable amounts from different protein substances.

It is very difficult to say to what extent all the above-mentioned carbon nuclei exist in the protein molecule. It is not inconceivable that in the hydrolysis certain carbon nuclei may be secondarily formed from others. We cannot exclude the possibility, as suggested by Loew,[1] that in the hydrolysis a marked atomic displacement perhaps occurs before cleavage, and for this reason two carbon nuclei, such as leucine and lysine, or tyrosine and phenylalanine, may be produced from the same atomic groupings, each according to the nature of the neighboring groups. Such a possibility cannot be entirely excluded, but it has been made rather improbable by recent investigations.

Even if we admit the above, still it is undoubtedly true that the chief cleavage products of the protein substances are amino-acids. Emil Fischer has shown that the amino-acids have the property of readily grouping together when water is split off and the amide group of one

[1] Loew, Die chem. Energie d. lebenden Zellen, München, 1898, and Hofmeister's Beiträge, 1.

amino-acid unites with the carboxyl group of the other. In accord with this behavior we can, as HOFMEISTER [1] has explained, but which was first proven by the epoch-making investigations of EMIL FISCHER, consider the proteins as chiefly formed by the condensation of amino-acids, where the amino-acids are united to each other by means of imino-groups according to the following scheme:



Closely connected with this conception is the question whether it is possible to prepare protein-like substances synthetically. In this connection we must mention that GRIMAUX and later also SCHÜTZENBERGER and PICKERING have been able to prepare substances, which in many properties are similar to the proteins, from various amino-acids either alone or mixed with other bodies such as biuret, alloxan, xanthine, or ammonia. Of special interest are the investigations of CURTIUS and his collaborators, in which they were able to prepare synthetically the so-called *biuret base* (triglycyl-glycine ethyl ester) and subsequently many other bodies which were related to the proteins. The most important work on the chaining of amino-acids has been performed by E. FISCHER [2] and his pupils. They have prepared a large number of complex bodies called *polypeptides*, which according to whether they contain two or more amino-acid groups united together, are called di-, tri-, tetrapeptides, etc. As examples of polypeptides we will mention—dipeptides: glycyl-alanine, leucyl tyrosine, propylalanine, diaminopropionic-acid dipeptide, lysyl-lysine, histidyl-histidine; leucyl-histidine; tripeptides: diglycyl-glycine, leucyl-alanyl-glycine, dileucylcystine; tetrapeptides: triglycyl-glycine, dileucyl-glycyl-glycine; pentapeptides: tetraglycyl-glycine, and leucyl-triglycyl-glycine; hexa- and heptapeptides: leucyl-tetraglycyl-glycine and leucyl-penta glycyl-glycine respectively. The most complex polypeptide thus far prepared is an octodecapeptide with 15 glycocoll and 3 leucine residues namely: *l*-leucyltriglycyl-*l*-leucyltriglycyl-*l*-leucyl-octoglycylglycine = $NH_2CH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_8.NHCH_2COOH$. with the sup-

[1] "Ueber den Bau des Eiweissmoleküls." Gesellsch. deutsch. Naturforscher und Aertze, Verhandl. 1902, and Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759.

[2] See Pickering, King's College, London, Physiol. Lab. Collect. Papers, 1897, which also cites Grimaux's work; also Journ. of Physiol., **18**, and Proceed. Roy. Soc., **60**, 1897; Schützenberger, Compt. rend., **106** and **112**; Curtius, Journ. f. prakt. Chem. (N. F.), **26** and **70**, and Ber. d. d. chem. Gesellsch., **37**; Fischer and collaborators, Untersuchungen über Aminosäuren, Polypeptide und Proteïne (1899–1906) and Ber. d. d. chem. Gesellsch., **39**, **40**, **41** and Annal. d. Chem. u. Pharm., **354**, **357**, **363**.

position that the amino-acids are here also combined together in the imide binding.

The large number of amino-acids isolated from the proteins make a large number of bindings possible. The number of possible combinations is still further increased by the fact that all the amino-acids with the exception of glycocoll contain at least one asymmetric carbon atom, and this leads to the possible formation of stereochemically different peptides. Thus in order to give a simple example, from two optically active amino acids, four different isomeric forms of dipeptides may occur, namely (if we designate the optical antipodes by *d*- and *l*-) *dd*, *ll*, *dl* and *ld*. Of these forms two can form a racemic dipeptide, thus *d*-alanyl-*d*-leucine + *l*-alanyl-*l*-leucine and *d*-alanyl-*l*-leucine + *l*-alanyl-*d*-leucine. As the proteins are optically active and on hydrolysis yield chiefly optically active amino-acids, those polypeptides which can be built up from the natural amino-acids of the proteins are of special importance in the study of the constitution of the proteins.

Most of the artificial polypeptides are constructed from monamino-mono-carboxylic acids, but polypeptides have also been prepared which contain diamino-acids or amino-dicarboxylic acids, and in this way the number of possible polypeptides becomes still greater. With an aminodicarboxylic acid such as aspartic acid, other amino-acids can be bound with one carboxyl group or with both, but also, if we start with asparagine, they can be anchored with the amide group. If we start from the acid amides we can also obtain a peptide which still contains the $CONH_2$ and on total hydrolysis yields NH_3, like most proteins. A polypeptide of this kind is the tripeptide glycyl-*l*-asparaginyl-*l*-leucine prepared by E. FISCHER and KOENIGS.

$$\begin{array}{l} NH_2CH_2CO.NH\underset{\displaystyle |\atop \displaystyle CH_2CONH_2}{CH}CO.NHCH(C_4H_9)COOH \end{array}$$

The methods used by E. FISCHER in the synthetical preparation of polypeptides are chiefly as follows:

The first dipeptide prepared by him, glycylglycine, he obtained from glycocoll ethyl ester which in water is transformed into a diketopiperazine, glycine anhydride, according to the following equation:

$$2(NH_2CH_2CO.O.C_2H_5) = 2C_2H_5OH + NH\left\langle\begin{array}{l} CH_2.CO \\ CO.CH_2 \end{array}\right\rangle NH.$$

By the action of dilute alkali upon this anhydride with the taking up of water the glycylglycine $NH_2CH_2CO.NHCH_2COOH$ is formed, and according to this principle other dipeptides can also be prepared.

Another method which has much greater application consists in the anchoring of an amino-acid to a halogen of an acid radical, for example, by the action of brompropionyl bromide or chloride upon glycocoll according to the following equation:

$CH_3CHBrCOCl + NH_2CH_2COOH = HCl + CH_3CHBrCO.NHCH_2COOH$

(brompropionyl glycine). On subsequent treatment with ammonia the halogen (Br) is replaced by NH_2 and the dipeptide alanylglycine

$$CH_3CHNH_2CO.NHCH_2COOH$$

is obtained. By the second action of brompropionylchloride and then treatment with NH_3 we introduce a new alanyl group and the tripeptide alanyl-alanyl glycine is prepared. By the action of a halogen derivative of an acid radical another amino-acid residue can be introduced, and the chain of amino groups can be thus extended.

The prolongation of the chain on the other side, namely, at the carboxyl, FISCHER has accomplished by chlorination of the amino-acids by special treatment with phosphorus pentachloride. The carboxyl is thus transformed into COCl, while the acid at the same time fixes a molecule of HCl, for example CH_3CHNH_2HCL

$$\begin{array}{c} | \\ COCl \end{array}$$

Just as in the case of the carboxyl group of an amino-acid, so also can a polypeptide or its halogen acyl combination be chlorinated and then combined with a new amino-acid, or a new peptide. As an example, FISCHER, from α-bromisocapronyldiglycyl glycine, first prepared α-bromisocapronyldiglycylglycyl chloride, and then with diglycylglycine he obtained the heptapeptide leucyl-pentaglycylglycine,

$$C_4H_9CH(NH_2)CO.(NHCH_2CO)_5.NHCH_2COOH.$$

For the various combinations of the optically active amino-acids to polypepteids it was important to possess methods of preparation of these amino-acids, and for this purpose FISCHER in many cases used the so-called WALDEN'S reversion. This consists in that one optically active amino-acid, for example the *l*-form, is transformed into the corresponding halogen fatty acid by the action of nitrosyl bromide, yielding the optical antipode the *d*-form. By the action of ammonia the *d*-amino-acid is now obtained which in the above-mentioned manner can be retransformed into the *l*-form. Thus from *d*-leucine we first obtain *l*-bromisocaproic acid and then by the action of ammonia *l*-leucine and in the preparation of the polypeptides the same occurs. Thus, for example, if by reversion *d*-leucine is changed first into *l*-bromisocapronyl chloride, if this last is combined with *l*-leucine, then we obtain the dipeptide *l*-leucyl-

l-leucine. On combination with diglycylglycine the tetrapeptide *l*-leucyl-diglycyl glycine is produced. WALDEN'S reversion does not take place with all amino-acids; other methods can also be used to obtain the optical antipodes, such as the preparation of the alkaloidal salts of the benzoyl or formyl combinations of the racemic amino-acids.

A comparison of the artificially prepared polypeptides with the proteins, and especially with the cleavage products of these last, the so-called proteoses and peptones, is of great interest in several respects, especially in connection with certain reactions. For instance there are several polypeptides which give the biuret reaction which is characteristic of the proteins in general, and also several (polypeptides containing tyrosine), which give MILLON'S reaction (see further on). The above-mentioned octodecapeptide is precipitated by phosphotungstic acid, tannin and ammonium sulphate; we also know tri- and pentapeptides containing tyrosine, which are very similar in properties to the proteoses.

The behavior of the polypeptides with proteolytic enzymes is of especially great interest. As far as known no artificial polypeptide is split by gastric juice. On the contrary there are many, as shown by FISCHER and especially by ABDERHALDEN and his collaborators, that are split by pancreatic juice or intestinal juice or by yeast-press juice, and by many enzymes occurring in animal tissues and fluids. The constitution of the polypeptides is of the greatest importance in this connection. Thus, for example, FISCHER and ABDERHALDEN,[1] have found with experiments with dog pancreatic juice that *d*-alanyl-*d*-alanine and *l*-leucyl-*l*-leucine are split, but not *d*-alanyl-*l*-alanine or *l*-leucyl-*d*-leucine. It seems that only those polypeptides which are constructed from amino-acids occurring in nature are hydrolyzable by enzymes. If a racemic dipeptide is in part hydrolyzable then it is composed in part of amino-acids occurring in nature. Only that part is split off and the cleavage takes place asymmetrically. If for example, we take the two racemic dipeptides from alanine and leucine mentioned on page 86, we find that of the two racemic bodies only one (*d*-alanyl-*l*-leucine+*l*-alanyl-*d*-leucine), which contains the combination *d*-alanyl-*l*-leucine, i.e., the two optical forms found in nature, is in part hydrolyzed while the other (*d*-alanyl-*d*-leucine+*l*-alanyl-*l*-leucine) is not attacked. The hydrolysis of the first racemic body is in this case referable, it seems, to the combination *d*-alanyl-*l*-leucine.

We will refer again, in Chapter IX, to this important and interesting enzymotic cleavage of the polypeptides. It is sufficient here to call atten-

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[1] Zeitschr. f. physiol. Chem., **51**. The numerous works of Abderhalden and collaborators cannot be here especially cited, but they may be found in Zeitschr. f. physiol. Chem. Bdd., **48**, **49**, **51**, **52**, **53**, **54**, **55**, **56**, **57**.

tion to the fact that a hydrolysis of polypeptides as well as proteins by the same enzymes with the splitting off of amino-acids is not sufficient evidence that the proteins contain the same form of amino-acid chains as the polypeptides.

We have also a very important support for this view in the fact that polypeptides occur in the cleavage products of the proteins. This is, to a certain extent, the reverse of the above-mentioned syntheses. After FISCHER and BERGELL were able to isolate from the cleavage products of silk fibroin a dipeptide in the form of an anhydride which seemed to be a glycylalanine, FISCHER and ABDERHALDEN[1] further studied this question, and by the partial hydrolysis of fibroin obtained not only the above-mentioned polypeptide, which they showed was glycyl-*d*-alanine anhydride, but also glycyl-*l*-tyrosine anhydride, and they established the identity by the correspondence with the synthetically prepared bodies. From elastin they obtained glycyl-*l*-leucine anhydride, *l*-leucyl-*d*-alanine anhydride, glycyl-*d*-valine anhydride and a dipeptide anhydride which yielded *d*-alanine and *l*-proline on hydrolysis. They have also shown the presence of other polypeptides among the hydrolytic products; we especially mention one tetrapeptide obtained from fibroin which consisted of 2 molecules glycocoll, 1 molecule *d*-alanine and 1 molecule *l*-tyrosine. This tetrapeptide gave the biuret reaction and also Millon's reaction. It was precipitated by tannic acid, by saturated ammonium sulphate solution, and by NaCl in the presence of free acid, and in many regards behaved like a proteose.

Polypeptides have also been found by other investigators in the hydrolytic cleavage products of proteins. Thus LEVENE with WALLACE and BEATTY isolated a glycyl-*l*-proline anhydride from gelatin after tryptic digestion, and LEVENE and BEATTY have shown the presence of a lysyl-glycyl peptide among the tryptic cleavage products of ovalbumin. OSBORNE and CLAPP[2] obtained from a plant protein, gliadin, a crystalline dipeptide which yielded phenylalanine and proline on cleavage.

We have therefore conclusive basis for the assumption that in the proteins, peptide bindings chiefly occur, i.e., a combination of the α-amino-acids by means of the imide binding. It is also possible that other bindings may occur, and FISCHER has also given expression to such a possibility. Besides the above-mentioned imide binding another kind must also without doubt exist in the proteins, namely, the anchoring of the urea-forming group (the guanidine residue) with the ornithin

[1] Fischer and Bergell, see Bicohem. Centralbl., I, p. 84. Fischer and Abderhalden, Sitz. Ber. d. k. Acad. d. Wissensch., **30** (1907) and Ber. d. d. chem. Gesellsch. **39** and **40**.

[2] Levene and Wallace, Zeitschr. f. physiol. Chem., **47**; Levene and Beatty, Ber. d. d. chem. Gesellsch., **39**, and Biochem. Zeitschr., **4**; Osborne and Clapp, Amer. Journ. of Physiol., **18**.

(diamino-valeric acid) by the imide binding. This imide binding is not, like the α-amino-acids, broken by trypsin, but rather by an enzyme, arginase, discovered by KOSSEL and DAKIN.[1]

If the proteins are considered as consisting chiefly of peptide-like complexes consisting of amino-acids united and containing also several NH_2 groups at the ends, it is readily understood that the proteins are amphoteric electrolytes, like the amino-acids, which form salts with bases as well as with acids and undergo hydrolytic dissociation. As we also accept the theory that the protein molecule contains a large number of COOH as well as NH_2 groups, it follows that the proteins may be polybasic acids as well as polyacidic bases. The different proteins act in this regard somewhat differently, thus the protamines are strongly basic while casein behaves strikingly acid, and others take a certain mean position. It is unfortunately impossible to base a classification of the proteins upon this behavior, as well as upon chemical constitution. The general properties, such as solubility and precipitation properties, are too uncertain to aid us, and especially as in the investigations of proteins we, as a rule, cannot decide whether we are dealing with a pure or with a contaminated substance, namely, with mixtures. Experience has shown that the solubility and precipitation property of the proteins is strongly influenced by the presence of other bodies, and under such circumstances a proper classification, as demanded by science, is impossible. On the other hand, a classification is important, and as the ones used up to the present time were based upon the solubilities and precipitation properties, we give the following schematic summary of the chief groups of protein bodies:

I. Simple Proteins.

A. TRUE ALBUMINOUS BODIES OR PROTEIDS

Albumins	*Seralbumin*, *Lactalbumin*, and others.
Globulins	*Fibrinogen*, *Serglobulins*, and others.
Phosphoproteins, (Nucleoalbumins)	*Ovovitellin*, *Casein*, and others.
Coagulated proteins.	
Histones.	
Protamines.	

[1] Zeitschr. f. physiol. Chem., 41.

B. Albuminoids or Albumoids.

Keratins.
Elastin.
Collagen and glutin.
Reticulin.
(Fibroin, Sericin, Coilin, Cornein, Spongin, Byssus, and others.)

C. Cleavage Products of True Albuminous Bodies.

Alkali and acid albuminates.
Proteoses, Peptones, Polypeptides.
(Amino-acids.)

II. Compound Proteins.

Glycoproteins ..... { *Mucin substances,* / *Ichthulin,* and others.

Nucleoproteins.

Chromoproteins ..... { *Hæmoglobin,* / *Hæmocyanin.*

As there are two classifications recognized by English-speaking scientists we will give the classifications adopted by the American Physiological Society and the Amercian Society of Biological Chemists and also the British Medical Association.

Classification adopted by the American Physiological Society and the American Society of Biological Chemists:

I. Simple Proteins.

A. **Albumins.**
B. **Globulins.**
C. **Glutelins.**
D. **Prolamins** (*Alcohol-soluble proteins*).
E. **Albuminoids.**
F. **Histones.**
G. **Protamines.**

II. Conjugated Proteins.

A. **Nucleoproteins.**
B. **Glycoproteins.**
C. **Phosphoproteins.**
D **Hæmoglobins.**
E. **Lecithoproteins.**

III. Derived Proteins.

1. Primary Protein Derivatives.

A. Proteans.
B. Metaproteins.
C. Coagulated proteins.

2. Secondary Protein Derivatives.

A. Proteoses.
B. Peptones.
C. Peptides.

Classification of proteins adopted by the British Medical Association:

I. Simple Proteins.

1. Protamines.
2. Histones.
3. Albumins.
4. Globulins.
5. Glutelins.
6. Alcohol-soluble proteins.
7. Scleroproteins.
8. Phosphoproteins.

II. Conjugated Proteins.

1. Glucoproteins.
2. Nucleoproteins.
3. Chromoproteins.

III. Products of Protein Hydrolysis.

1. Infraproteins.
2. Proteoses.
3. Peptones.
4. Polypeptides.

To this summary must be added that we often find in the investigations of animal fluids and tissues protein substances which do not fall in with the above schemes, or are classified only with difficulty. At the same time it must be remarked that bodies will be found which seem to rank between the different groups, hence it is very difficult to sharply divide these groups.

I. Simple Proteins.

A. True Albuminous Bodies.

The albuminous bodies are never-failing constituents of the animal and vegetable organisms. They are especially found in the animal body, where they form the solid constituents of the muscles and of the blood-serum, and they are so generally distributed that there are only a few animal secretions and excretions, such as the tears, the perspiration, and perhaps the urine, in which they are entirely absent or occur only in traces.

All albuminous bodies contain *carbon, hydrogen, nitrogen, oxygen,* and *sulphur*;[1] a few contain also *phosphorus. Iron* is generally found in traces in their ash, and it seems to be a regular constituent of a certain group of the albuminous bodies, namely, the nucleoalbumins. The composition of the different albuminous bodies varies a little, but the variations are within relatively close limits. For the better-studied animal albuminous bodies the following composition of the ash-free substance has been found:

C	50.6 —54.5	per cent.
H	6.5 — 7.3	"
N	15.0 —17.6	"
S	0.3 — 2.2	"
P	0.42— 0.85	"
O	21.50—23.50	"

The animal proteids are odorless, tasteless, and ordinarily amorphous. The crystalloid spherules (*Dotterplättchen*) occurring in the eggs of certain fishes and amphibians, do not consist of pure proteids, but of albuminous bodies containing large amounts of lecithin, which seem to be combined with mineral substances. Crystalline proteids[2] have been prepared from the seeds of various plants, and crystallized animal proteids (see seralbumin and ovalbumin, Chapters VI and XIII) can be readily prepared. In the dry condition the proteids appear as white powders, or when in thin layers as yellowish, hard, transparent plates. A few are soluble in water, others only soluble in salt or faintly alkaline or acid solutions, while others are insoluble in these solvents. Solutions of proteids are optically active and turn the plane of polarized light to the left. All proteids when burned leave an ash, and it is therefore ques-

[1] See footnote 3, p. 78.

[2] See Maschke, Journ. f. prakt. Chem., **74**; Drechsel, *ibid.* (N. F.), **19**; Grübler, *ibid.* (N. F.), **23**; Ritthausen, *ibid.* (N. F.), **25**; Schmiedeberg, Zeitschr. f. physiol. Chem., **1**; Weyl., **1**; *ibid.*, **1**.

tionable whether there exists any proteid body which is soluble in water without the aid of mineral substances. Nevertheless it has not been thus far successfully proven that a native proteid body can be prepared perfectly free from mineral substances without changing its constitution or its properties.[1]

As previously stated, the albuminous bodies are amphoteric electrolytes, and are polyacidic bases as well as polybasic acids. The base- and acid-combining powers of various proteids are different, and the maximum acid-combining power may perhaps also be used in the differentiation of the various proteids (COHNHEIM, ERB, and others).

The acid-combining power of the proteins has been studied by means of physical methods by SJÖQVIST, BUGARSKY, and LIEBERMANN and with the aid of chemical methods by SPIRO and PEMSEL, ERB, COHNHEIM and KRIEGER, and v. RHORER. The methods pursued by COHNHEIM and KRIEGER consisted in precipitating the proteid from acid solution (HCl) with an alkaloid reagent (calcium phosphotungstate). The reaction takes place as follows: proteid hydrochloride + calcium phosphotungstate = proteid phosphotungstate + calcium chloride. The acid remaining in the filtrate was determined, and when this quantity was subtracted from the known original amount in the proteid solution, the difference represented the acid combined with the proteid. If sodium picrate or potassium-mercuric iodide is used instead of the phosphotungstate we have, according to v. RHORER,[2] a method which is the best of all heretofore suggested.

The proteids can be salted out from their neutral solutions by neutral salts (NaCl, Na_2SO_4, $MgSO_4$, $[NH_4]_2SO_4$, and many others) in sufficient concentrations. By this salting out the properties remain unchanged and the process is reversible, as on diminishing the concentration of the salt the precipitate redissolves. The various proteids act in an essentially different manner toward the same salt, and also for one and the same proteid the behavior toward different neutral salts is different, as some cause a precipitate, while others on the contrary do not precipitate.

The behavior of various proteids with one and the same salt, such as $MgSO_4$ or $(NH_4)_2SO_4$, is often made use of in the isolation of the proteid, and special methods of separation are based upon fractional precipitation. HASLAM[3] has recently shown that these methods may lead to great errors, and give good results only under special conditions.

The conditions are different from those of salting out, when the proteid solution is precipitated by salts of the heavy metals. Here the precipitates (often called metallic albuminates) are not true combina-

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[1] See E. Harnack, Ber. d. d. chem. Gesellsch., **22**, **23**, **25**, and **31**; Werigo, Pflüger's Archiv, **48**; Bülow, *ibid.*, **58**; Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903.

[2] Pflüger's Arch., **90**. In regard to the literature on this subject see Cohnheim, Chemie der Eiweisskörper, 2. Aufl., pp. 107–109.

[3] See Cohnheim, Chemie der Eiweisskörper, 2. Aufl., 1904, pp. 144-148; Pinkus, Journ. of Physiol., **27**; Pauli, Hofmeister's Beiträge, **3**, p. 225; Halsam, Journ. of Physiol., **32**.

tions in constant proportions, but are rather to be considered as loose adsorption compounds of the proteid with the salt.[1] These reactions are irreversible in so far that dilution with water or removal of the salt by means of dialysis does not restore the unchanged proteid. On the other hand the precipitate, at least in certain cases may be redissolved in an excess of the salt solution or of the proteid solution, and in this sense the process is a reversible one.

The precipitation of proteids and also other soluble proteins by salts stands in close relation to their colloidal nature, and in this connection we refer to what has been said in Chapter II. The proteids do not as a rule diffuse through animal membranes, or only to a very slight extent, and hence have in most cases a pronounced colloidal nature in GRAHAM'S sense. They belong to the hydrophile colloids; their solutions show properties in common with those of typical colloids and also true solutions. Certain of them, especially the peptones and a few proteoses, which will be discussed later, seem to occupy an intermediate position, as their solutions are characterized by a lesser viscosity and greater diffusibility and filtration ability, are not readily precipitable by alcohol or coagulable by heat, and are only slightly precipitable by salts.

The solutions (or suspensions) of proteids in water, the proteid hydrosols, are converted by various means into proteid hydrogels. Of these means we must specially mention the following: flocking out with salts, precipitation with alcohol, gelatinization of a gelatin solution on cooling, and coagulation by the action of enzymes or heat.

Those proteids which occur, according to the common views, preformed in the animal fluids and tissues, and which have been isolated from these by indifferent chemical means without losing their original properties, are called *native proteids.* New modifications having other properties can be obtained from the native proteids by heating, by the action of various chemical reagents such as acids, alkalies, alcohol, and others, as well as by proteolytic enzymes. These new proteids are called *modified* (" *denaturierte* ") *proteids,* to differentiate them from the native proteids.

The precipitation with alcohol is a reversible reaction, as the precipitate redissolves on subsequent dilution with water. The proteids are changed by the action of alcohol, some readily and quickly, others with difficulty and very slowly; the proteid then becomes insoluble in water and is modified.

On heating a solution of a native proteid it is modified at a different temperature for each different proteid. With proper reaction and other

[1] See Galeotti, Zeitschr. f. physiol. Chem., 40, 42, 44, and 48 and Bonamartini and Lombardi, *ibid.*, 58.

favorable conditions, for instance in the presence of neutral salts, most proteids can in this way be precipitated in a solid form as coagulated proteid. The hydrosol is converted into hydrogel, but as a modification takes place, this process is irreversible. The various temperatures at which coagulation of different proteids occurs in neutral solutions containing salt have in many cases given us good means for detecting and separating proteids. The views in regard to the use of these means are somewhat divided.[1]

A modification can be brought about also by the action of acids, alkalies, or salts of the heavy metals, in certain cases by water alone, and also by the action of alcohol, chloroform,[2] and ether, by violent shaking (RAMSDEN [3]), etc.

An adsorption of proteids by a suspension colloid such as silicic acid, colloidal ferric hydroxide and kaolin, can easily take place, and indeed the proteid of a solution can be removed by the use of colloidal ferric hydroxide or shaking with kaolin (RONA and MICHAELIS [4]). That the proteids can serve as preventives in the precipitation of suspension colloids has been mentioned in Chapter II in speaking of the gold equivalent. In the same manner a mastic suspension is protected from the precipitating action of an electrolyte by an excess of a proteid solution, while the reverse may be brought about, namely, a proteid solution can be precipitated by a large quantity of mastic emulsion in the presence of a proportionately small amount of electrolyte. The method for the removal of proteid from solutions, as suggested by MICHAELIS and RONA,[5] is based upon this behavior.

Proteid solutions free from electrolytes have, according to PAULI,[6] no electric charge, hence they do not migrate in an electric field. On the addition of a trace of acid the proteid becomes electro-positive and moves toward the cathode, while on the addition of alkali it becomes electro-negative and wanders in the electric current to the anode. In the natural

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[1] See Halliburton, Journ. of Physiol., **5** and **11**; Corin and Berard, Bull. de l'Acad. roy. de Belg., **15**; Haycraft and Duggan, Brit. Med. Journ., 1890, and Proc. Roy. Soc. Edin., 1889; Corin and Ansiaux, Bull. de l'Acad. roy. de Belg., **21**; L. Frédericq, Centralbl. f. Physiol., **3**; Haycraft, *ibid.*, **4**; Hewlett, Journ. of Physiol., **13**; Duclaux, Annal. Institut Pasteur, **7**. In regard to the relationship of the neutral salts to the heat coagulation of albumins see also Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München, 1897; Pauli, Pflüger's Arch., **78**.

[2] See Salkowski, Zeitschr. f. physiol. Chem., **31**; Fr. Krüger, Zeitschr. f. Biologie, **41**; Loew and Aso, Bull. Coll. Agric., Tokio, **4**.

[3] Ramsden, Zeitschr. f. physik. Chem., **47** and Arch. f. (anat. u.) Physiol., 1894.

[4] Biochem. Zeitschr., **5**.

[5] Biochem. Zeitschr., **2**, **3** and **4**.

[6] Hofmeister's Beiträge, **7**.

fluids like the blood-serum it is electro-negatively charged, which fact can be explained by the presence of an excess of OH ions.

The determination of the molecular weight of the proteids has been attempted by various methods which are more or less uncertain.[1] There is no doubt that the molecular weight of the proteids is very high, but the statements about the size vary very considerably. For the true proteids thus far investigated, values ranging from 4000—6000—10,000 have been found.

The general reactions for the proteids are very numerous, but only the most important will be given here. To facilitate the study of these, they have been divided into the two following groups. It must be remarked that the precipitation reactions are not only applicable for the soluble true proteids but also, more or less, for other soluble proteins in general. The color reactions are applicable to all soluble or insoluble proteins with few exceptions, which will be mentioned later.

Precipitation Reactions of the Proteid Bodies.

1. *Coagulation Test.* An alkaline proteid solution does not coagulate on boiling, and a neutral solution only partly and incompletely; the reaction must therefore be acid for coagulation. The neutral liquid is first boiled and then the proper amount of acid added carefully. A flocculent precipitate is formed, and with proper technique the filtrate should be water-clear. If dilute acetic acid be used for this test, the liquid must first be boiled and then 1, 2, or 3 drops of acid added to each 10–15 cc., depending on the amount of proteid present, and boiled before the addition of each drop. If dilute nitric acid (25 per cent) be used, then to 10–15 cc. of the previously boiled liquid 15–20 drops of the acid must be added. If too little nitric acid be added, a soluble combination of the acid and proteid is formed, which is precipitated by more acid. A proteid solution containing a small amount of salts must first be treated with about 1 per cent NaCl, since the heating test may fail, especially on using acetic acid, in the presence of only a slight amount of proteid.

2. *Precipitation by Alcohol.* The solution must not be alkaline, but must be either neutral or faintly acid. It must, at the same time, contain sufficient quantity of neutral salts.

3. *Neutral Salts,* such as Na_2SO_4 or NaCl, when added to saturation precipitate certain proteids but not others. *Ammonium sulphate* when dissolved to saturation in the liquid is considered as the general precipitant for proteids. In the presence of free acetic or hydrochloric acid the above-mentioned salts, NaCl or Na_2SO_4, in sufficient concentration, are also general precipitants for the proteids.

[1] See especially F. N. Schulz, Die Grösse des Eiweissmolecüle, Jena, 1903.

4. *Precipitation by Metallic Salts* such as copper sulphate, ferric chloride, neutral and basic lead acetate (in small amounts), mercuric chloride and others. On this is based the use of proteids as antidotes in poisoning with metallic salts.

5. *Precipitation by Mineral Acids at Ordinary Temperatures.* The proteids are precipitated by the three ordinary mineral acids in proper amounts, but not by orthophosphoric acid. If nitric acid be placed in a test-tube and the proteid solution be allowed to flow gently thereon, a white opaque ring of precipitated proteid will form where the two liquids meet (HELLER'S albumin test).

6. *Precipitation by the so-called Alkaloid Reagents.* To these belong the precipitation by *metaphosphoric acid* and by *hydroferrocyanic acid*, which is carried out by the aid of potassium ferrocyanide in a liquid containing acetic acid; precipitation by *phosphotungstic acid* or *phosphomolybdic acid* in the presence of free mineral acids; precipitation by *potassium-mercuric iodide* or *potassium-bismuth iodide* in solutions acidified with hydrochloric acid; precipitation by *tannic acid* in acetic acid solutions. The absence of neutral salts or the presence of free mineral acids may prevent the appearance of the precipitate, but after the addition of a sufficient quantity of sodium acetate the precipitate will in both cases appear; precipitation by *picric acid* in solutions acidified by organic acids. Proteids are also precipitated by *trichloracetic acid* in 2–5 per cent solutions, by *phenol, salicyl sulphonic acid, nucleic acid, taurocholic acid* and by *chondroitin sulphuric acid* in acid solutions.

Color Reactions for Proteid Bodies.

1. *Millon's Reaction.*[1] A solution of mercury in nitric acid containing some nitrous acid gives a precipitate with proteid solutions which at the ordinary temperature is slowly, but at the boiling-point more quickly, colored red; and the solution may also be colored a feeble or bright red. Solid albuminous bodies, when treated by this reagent, give the same coloration. This reaction, which depends on the presence of the aromatic group in the proteid, is also given by tyrosine and other monohydroxyl benzene derivatives. According to O. NASSE[2] it is best to use a solution of mercuric acetate which is treated with a few drops of a 1 per cent solution of potassium or sodium nitrite; previous to use

[1] The reagent is prepared in the following way: 1 pt. mercury is dissolved in 2 pts. nitric acid (of sp.gr. 1.42), first cold and then warmed. After complete solution of the mercury add 1 volume of the solution to 2 volumes of water. Allow this to stand a few hours and decant the supernatant liquid.

[2] See O. Nasse, Sitzungsb. d. Naturforsch. Gesellsch. zu Halle, 1879, and Pflüger's Arch., 83; see also Vaubel and Blum, Journ. f. prakt. Chem. (N. F.), 57.

a few drops of acetic acid are added. 2. *Xanthoproteic Reaction.* With strong nitric acid the albuminous bodies give, on heating to boiling, yellow flakes or a yellow solution. After making alkaline with ammonia or alkalies the color becomes orange-yellow. 3. *Adamkiewicz's Reaction.* If a little proteid is added to a mixture of 1 vol. concentrated sulphuric acid and 2 vols. glacial acetic acid a reddish-violet color is obtained slowly at ordinary temperatures, but more quickly on heating. According to HOPKINS and COLE [1] this reaction takes place only on using glacial acetic acid containing glyoxylic acid. According to them it is better to use a solution of glyoxylic acid, which can be readily prepared by adding sodium amalgam to a concentrated solution of oxalic acid and filtering after the discharge of the gas. A dilute aqueous solution of the acid or some of the solid acid is added to the proteid solution and sulphuric acid allowed to flow down the side of the test-tube, when the reddish-violet color will appear at the point of contact of the two liquids. Gelatin does not give this reaction.

A similar color is obtained, according to ACREE, with *formaldehyde.* About 0.1 gm. of the substance is treated with 0.1 cc. formaldehyde solution (1:5000) and then after 2–3 minutes carefully treated with 0.5 cc. sulphuric acid. At the boundary of the two layers a violet coloration occurs. According to ROSENHEIM [2] this reaction occurs in the presence of oxidizing substance only, and further investigations into this reaction are necessary.

As further color reactions we will mention: 4. *Biuret Test.* If a proteid solution be first treated with caustic potash or soda and if then a dilute copper-sulphate solution be added drop by drop, first a reddish then a reddish-violet, and lastly a violet-blue, color is obtained. 5. Proteids are soluble on heating with *concentrated hydrochloric acid,* producing a violet color, and when they are previously boiled with alcohol and then washed with ether (LIEBERMANN [3]) they give a beautiful blue solution. This blue color is due, according to COLE,[4] to a contamination of the ether with glyoxylic acid, which reacts with the tryptophane groups split off by the hydrochloric acid. The violet color obtained with proteins not purified with ether is also considered as a tryptophane reaction, produced from the furfurol formed by the action of the concentrated hydrochloric acid upon the proteid. Reaction 6 with *concentrated sulphuric acid* and *sugar* (in small quantities) is explained in the same way. The beautiful red coloration is connected with the formation of furfurol from the sugar. 7. With *p*-dimethylaminobenzal-

[1] Proceed. Roy. Soc., 68.

[2] Acree, Amer. chem. Journ., **37**; Rosenheim, Chem. Centralbl., 1907, p. 1809. See also Dakin, Journ. of Biol. Chem., **2**.

[3] Centralbl. f. d. med. Wissensch., 1887.

[4] Journ. of Physiol., **30**.

dehyde and concentrated sulphuric acid the proteids give a beautiful reddish-violet or deep-violet coloration (O. NEUBAUER and E. ROHDE[1]).

Many of these color reactions are obtained as shown by SALKOWSI,[2] by the aromatic or heterocyclic cleavage products of the proteids. MILLON'S reaction is given only by the substances of the phenol group; the XANTHOPROTEIC reaction by the phenol group and skatol or the indol group. LIEBERMANN'S reaction depends, according to COLE, upon the indol group, and the reactions with sulphuric acid and sugar (COLE) and with diamethylaminobenzaldehyde (ROHDE) are also caused by this group. ADAMKIEWICZ'S reaction is given only by the bodies of the indol group. The biuret reaction is not only given by protein substances, but also by many other bodies. According to H. SCHIFF[3] this reaction occurs with those bodies containing amino groups, $CONH_2$, $CSNH_2$, $C(NH)NH_2$ or also CH_2NH_2, united either directly by their carbon atoms or by means of a third carbon or nitrogen atom. As examples of such bodies we can mention several diamines or aminoamides, such as oximide, biuret, glycinamide, α- and β-aminobutyramide, aspartic-acid amide, etc., although we are not clear as to the conditions necessary for the bringing about of this reaction. The biuret reaction alone is therefore no proof as to the protein nature of a substance—for example, urobilin gives a very similar color reaction—and a protein substance can still retain its protein nature, as by the action of nitrous acid or by a splitting off of ammonia, although it does not give the biuret reaction.

The delicacy of the various reagents differs for the different proteids, and on this account it is impossible to give the degree of delicacy for each reaction for all proteids. Of the precipitation reactions, HELLER'S test (if we eliminate the peptones and certain proteoses) is recommended in the first place for its delicacy, though it is not the most delicate reaction, and because it can be performed so easily. Among the precipitation reactions, that with basic lead acetate (when carefully and exactly executed) and with alcohol and the reactions given under 6, are the most delicate. The color reactions 1 to 4 show great delicacy in the order in which they are given.[4]

No proteid reaction is in itself characteristic, and, therefore, in testing for proteids one reaction is not sufficient, but a number of precipitation and color reactions must be employed.

For the quantitative estimation of coagulable proteids the determination by boiling with acetic acid can be performed with advantage, for by operating carefully, it gives exact results. Treat the proteid solution with a 1–2 per cent common-salt solution, or if the solution contains large amounts of proteid dilute with the proper quantity of the above salt solution, and then carefully neutralize with acetic acid. Now determine the quantity of acetic acid necessary to completely precipitate the proteids in small measured portions of the neutralized liquid which

[1] Zeitschr. f. physiol. Chem., 44.

[2] *Ibid.*, 12.

[3] Ber. d. d. chem. Gesellsch., 29 and 30.

[4] In regard to the precipitation and coloration reactions of proteids with aniline dyes see Heidenhain, Pflüger's Arch., 90, 96.

have previously been heated on the water-bath, so that the filtrate does not respond to HELLER'S test. Now warm a larger weighed or measured quantity of the liquid on the water-bath, and add gradually the required quantity of acetic acid, with constant stirring, and continue heating for some time. Filter, wash with water, extract with alcohol and then with ether, dry, weigh, incinerate, and weigh again. With proper work the filtrate should not give HELLER'S test. This method serves in most cases, and especially so in cases where other bodies are to be quantitatively estimated in the filtrate.

The precipitation by means of alcohol may also be used in the quantitative estimation of proteids. The liquid is first carefully neutralized, treated with some NaCl if necessary, and then alcohol added until the solution contains 70–80 vol. per cent anhydrous alcohol. The precipitate is collected on a filter after 24 hours, extracted with alcohol and ether, dried, weighed, incinerated, and again weighed. This method is only applicable to liquids which do not contain any other substances, like glycogen, which are insoluble in alcohol.

In both of these methods small quantities of proteid may remain in the filtrates. These traces may be determined as follows: Concentrate the filtrate sufficiently, remove any separated fat by shaking with ether, and then precipitate with tannic acid. Approximately 63 per cent of the tannic-acid precipitate, washed with cold water and then dried, may be considered as proteid.

In many cases good results may be obtained by precipitating all the proteid with tannic acid and determining the nitrogen in the washed precipitate by means of KJELDAHL'S method. On multiplying the quantity of nitrogen found by 6.25 we obtain the quantity of proteid.

The removal of proteids from a solution may in most cases be performed by boiling with acetic acid. Small amounts of proteid which remain in the filtrates may be separated by boiling with freshly precipitated lead carbonate or with ferric acetate, as described by HOFMEISTER.[1] If the liquid cannot be boiled, the proteid may be precipitated by the very careful addition of lead acetate, or by the addition of alcohol. If the liquid contains substances which are precipitated by alcohol, such as glycogen, then the proteid may be removed by the alternate addition of potassium-mercuric iodide and hydrochloric acid (see Chapter VIII, on Glycogen Estimation), or by trichloracetic acid as suggested by OBERMAYER and FRÄNKEL.[2] Recently MICHAELIS and RONA have suggested a method for the removal of proteids by using kaolin, colloidal ferric hydrate or a mastic emulsion. The principle of these methods has already been given on page 96 and in regard to the practical execution of the method we refer to the works there cited.

In the precipitation of proteid as well as the quantitative estimation by means of heat, it must be borne in mind, as shown by SPIRO,[3] that several nitrogenous substances, such as piperidine, pyridine, urea, etc., disturb the coagulation of the proteids.

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[1] Zeitschr. f. physiol. Chem., 2 and 4.

[2] Obermayer, Wien. med. Jahrb., 1888; Fränkel, Pflüger's Arch., 52 and 55.

[3] Zeitschr. f. physiol. Chem., 30.

Synopsis of the Most Important Properties of the Different Groups of Albuminous Bodies.

As it is not possible to base the classification of the different proteid groups according to their constitution, we are obliged to make use of their different solubilities and precipitation properties in their general characterization. As there exist no sharp differences between the various groups in this regard it is impossible to draw a sharp line between them.

Albumins. These bodies are soluble in water and are not precipitated by the addition of a little acid or alkali. They are precipitated by the addition of large quantities of mineral acids or metallic salts. Their solution in water coagulates on boiling in the presence of neutral salts, but a weak saline solution does not. If NaCl or $MgSO_4$ is added to saturation to a neutral solution in water at the normal temperature or at 30° C. no precipitate is formed; but if acetic acid is added to this saturated solution the albumins readily separate. When ammonium sulphate is added to one-half saturation the albumin solutions are not precipitated at ordinary temperatures. Of all the native proteids the albumins are the richest in sulphur, containing from 1.6 per cent to 2.2 per cent. So far as they have been investigated they do not yield any glycocoll on acid hydrolysis.

Globulins. These substances are, as a rule, insoluble in water, but dissolve in dilute neutral salt solutions. The globulins are precipitated unchanged from these solutions by sufficient dilution with water, and on heating they coagulate. The globulins dissolve in water on the addition of very little acid or alkali, and on neutralizing the solvent they precipitate again. The solution in a minimum amount of alkali is precipitated by carbon dioxide, but the precipitate may be redissolved by an excess of the precipitant. The neutral solutions of the globulins containing salts are partly or completely precipitated on saturation with NaCl or $MgSO_4$ in substance at normal temperatures, depending upon the kind of globulin. The globulins are completely precipitated by half-saturating with ammonium sulphate. The globulins contain an average amount of sulphur generally not below 1 per cent. As a difference between the albumins and globulins the latter yield glycocoll among the hydrolytic cleavage products.

According to J. Starke [1] the globulins are not soluble in dilute salt solutions, but form alkali proteid compounds whose solubility in salts is brought about by

[1] Zeitschr. f. Biologie, **40** and **42**. In regard to the various views on this subject see Wolff and Smits, *ibid.*, **41**; Osborne, l. c. Hammarsten, Ergebnisse der Physiologie, Jahrg. I, Abt. 1.

an increase in the free OH ions produced by the salts. This view is not tenable for several globulins, and seems in fact not to be well founded.

That a sharp line cannot be drawn between the albumins and globulins follows from the fact that the albumins can be converted into globulins. The possibility of a conversion of ovalbumin into globulin is based upon the observations of STARKE. That a transformation of seralbumin into serglobulin with the aid of the weak action of alkali in the warmth, with the splitting off of sulphur, can take place, has been more conclusively shown by MOLL[1] by experimenting with blood-serum as well as with crystalline seralbumin. According to MOLL, first pseudoglobulin is formed from the seralbumin, and then euglobulin (see Chapter VI). The artificial globulins thus obtained had the same sulphur content and properties as the natural products.

It is evident that we are here dealing with a change of the external properties of the albumins to a greater similarity to those of the globulins, and not with a true transformation of the albumin into globulin. This follows from the fact that by the action of weak alkali upon albumin, which is free from glycocoll, we do not obtain globulin which contains glycocoll. This is an instructive example of the subordinate importance the solubility and precipitation properties have in the differentiation of various groups of proteids.

It is just as difficult to draw a sharp line between the globulins and albuminates as it is between the globulins and albumins. Several globulins are very readily changed by the action of very little acid, as also by standing under water when in a precipitated condition, into albuminates, and then become insoluble in neutral salt solutions. OSBORNE,[2] who has closely studied this property in connection with edestin (from hempseed), considers the globulin, "globan," which has been made insoluble in salt solution, as an intermediate step in the formation of the albuminate which is produced by the hydrolytic action of the H ions of water or of the acid.

Phosphoproteins are a group of phosphorized proteids which occur extensively in the animal and plant kingdoms and which include the *nucleoalbumins* and the little-studied *lecithalbumins*.

Nucleoalbumins. These proteids behave like rather strong acids, are nearly insoluble in water, but dissolve easily with the aid of a little alkali and, in the entire absence of lecithin, contain also phosphorus. Certain of the nucleoalbumins resemble the globulins by their solubility and precipitation properties. Others resemble the albuminates, but differ from both of these groups by containing phosphorus. They stand

[1] Hofmeister's Beiträge, **1** and **7**.

[2] Zeitschr. f. physiol. Chem., **33**.

close to the nucleoproteins by their content of phosphorus, but differ from these in not yielding any purine bases on cleavage. It has not yet been found possible to obtain from the neucleoalbumins any proteid-free pseudonucleic acids corresponding to the nucleic acids, but only acids rich in phosphorus, which always give the proteid reactions (LEVENE and ALSBERG, SALKOWSKI, REH [1]). For this reason the nucleoalbumins cannot be classed as compound proteins. In peptic digestion a proteid rich in phosphorus can be split off from most nucleoalbumins, and this has been called *para-* or *pseudonuclein*. The claim made that the pseudonuclein is a combination of proteid with metaphosphoric acid has been shown to be incorrect by the investigations of GIERTZ.[2] The nucleoalbumins always seem to contain some iron.

The separation of pseudonuclein in peptic digestion is no doubt characteristic of the nucleolabumin group, but the non-appearance of the pseudonuclein precipitate does not entirely exclude the presence of a nucleoalbumin. The extent of such a formation is dependent upon the intensity of the pepsin digestion, the degree of acidity, and the relation between the nucleoalbumins and the digestive fluids. The separation of a pseudonuclein may, as shown by SALKOWSKI, not occur even in the digestion of ordinary casein, and WRÓBLEWSKI did not obtain any pseudonuclein at all in the digestion of the casein from human milk. WIMAN [3] has also shown in the digestion of vegetable nucleoalbumin that the obtainment of considerable pseudonuclein or none is dependent upon the way in which the digestion is performed. The most essential characteristic of this group of proteids is that they contain phosphorus, and that the purine bases are absent in their cleavage products.

The nucleoalbumins are often confounded with nucleoproteins and also with phosphorized glucoproteins. From the first class they differ by not yielding any purine bases when boiled with acids, and from the second group by not yielding any reducing substance on the same treatment. The best studied member of this group is the casein of milk, which will be discussed in detail in a subsequent chapter (XIV).

Lecithalbumins. In the preparation of certain protein substances, products are often obtained containing lecithin, and this lecithin can be removed only with difficulty or incompletely by a mixture of alcohol and ether. Ovovitellin (Chapter XIII) is such a protein body containing considerable lecithin, and HOPPE-SEYLER considers it a combination of proteid and lecithin. Similar substances occur in fish-eggs. These last lecithalbumins often have the solubilities of the globulins,

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[1] Levene and Alsberg, *ibid.*, **31**; Salkowski, *ibid.*, **32**; Levene, *ibid.*, **32**; A. Reh, Hofmeister's Beiträge, **11**.

[2] Giertz, Zeitschr. f. physiol. Chem., **28**.

[3] Salkowski, Pflüger's Arch., **63**; Wróblewski, Beiträge zur Kenntnis des Frauenkaseïns, Inaug.-Diss., Bern, 1894; Wiman, Upsala Läkaref. Förh. (N. F.), **2**.

and are readily soluble in dilute salt solutions. The behavior of the nucleoalbumin of the eggs of the perch shows how easily this solubility may be changed. This nucleoalbumin, which contains considerable amounts of lecithin, is readily soluble in dilute NaCl solution, but at ordinary temperatures it is changed by 0.1 per cent HCl nearly instantaneously and without splitting off lecithin, so that it becomes insoluble in dilute salt solutions (HAMMARSTEN). LIEBERMANN [1] has obtained proteids containing lecithin as an insoluble residue on the peptic digestion of the mucous membrane of the stomach, liver, kidneys, lungs, and spleen. He considers them as combinations of proteid and lecithin and calls them *lecithalbumins*. Further investigation of these bodies is desirable.

MAYER and TERROINE [2] have shown that from lecithin emulsified in water and a dialyzed solution of ovalbumin or dialyzed blood serum a precipitate can be obtained which has some similarity to the lecithalbumins, but which in other respects is so strikingly different that we are not justified in calling this precipitate lecithalbumin.

Nothing characteristic has thus far been found which differentiates this group from others in the quantity of amino-acids split off on hydrolysis. The members of this group differ essentially among themselves, e.g., vitellin yields glycocoll while casein does not.

In order to give a review of the three above-mentioned groups of proteids we give (page 106) a tabulation of the amounts of the amino-acids obtained on cleavage, but we must bear in mind that the figures, because of the difficulty in the quantitative estimation, are not quite exact, but must be considered as minimum values. As a representative of the globulin group we give fibrin, which is a coagulated globulin; and as representative of the phosphoprotein group, ovovitellin, although not quite pure. The results are based on 100 parts of the substance.

The proteins occurring in the plant kingdom correspond in part to the above-described three groups of animal proteids. Among these the globulins are especially represented, and as an example we will specially mention the crystalline *edestin*, occurring in the hemp-seed. It is not clear whether the phosphorized plant proteids contain their phosphorus as impurities or whether they are the same as the animal phosphoproteins. There is no doubt that certain vegetable proteids cannot be classified in the above groups, namely, *gliadin* of the wheat and *zein* of the corn kernel, which are proteins soluble in alcohol. They are also characterized by not yielding any lysine.

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[1] Hoppe-Seyler, Med. chem. Untersuch., 1868; also Zeitschr. f. physiol. Chem., **13**, 479; Hammarsten, Skand. Arch. f. Physiol., **17**; Liebermann, Pflüger's Archiv, **50** and **54**.

[2] Compt. rend. soc. biol., **62**.

	Lact-albumin.[1]	Ser-albumin.[4]	Ov-albumin.[5]	Ser-globulins.[2]	Fibrin.[11]	Casein.[2]	Vitellin.[10]
Glycocoll	0.0	0.0	0.0	3.5	3.0	0.0	1.1
Alanine	2.5	2.7	2.1	2.2	3.6	0.9	+
Valine	0.9	....	} 17[6]	+	1.0	1.0	2.4
Leucine	....	20.0	6.1	18.7	15.0	10.5	11.0
Serine	....	0.6	1.1	....	0.8	0.23	....
Aspartic acid	1.0	3.1	1.5	2.5	2.0	1.2	0.5
Glutamic acid	10.1	7.7[2]	8.0	8.5	10.4	11.1	12.2
Cystin	....	2.53[3]	0.3[3]	1.51[3]	1.17[3]	0.07[3]	....
Phenylalanine	2.4	3.1	4.4	3.8	2.5	3.2	2.8
Tyrosine	0.85	2.1	2.4[3]	2.5	3.5	4.5	1.6
Proline	4.0	1.04	2.25	2.8	3.6	3.1	3.3
Oxyproline	....	....	....	....	....	0.25	....
Tryptophane	....	....	....	....	....	1.5[9]	....
Histidine	....	....	....	....	....	2.59[8]	....
Arginine	....	....	....	....	3.0[7]	4.84[8]	....
Lysine	....	....	....	....	4.0[7]	5.80[8]	....

[1] Abderhalden and H. Pribram, Zeitschr. f. physiol. Chem., **21**.
[2] Abderhalden, Lehrb. d. physiol. Chem., 1909.
[3] K. Mörner, Zeitschr. f. physiol. Chem., **34**.
[4] Abderhalden, *ibid.*, **37**.
[5] Abderhalden and Pregl., *ibid.*, **46**.
[6] Levene and Beatty, Bicohem. Zeitschr., **4**.
[7] Kutscher, Endprodukte der Trypsin Verdauung, Habit. Schrift., Marburg, 1899.
[8] E. Hart, Zeitschr. f. physiol. Chem., **33**.
[9] Hopkins and Cole, Journ. of Physiol., **27**.
[10] Abderhalden and Hunter, Zeitschr. f. physiol. Chem., **48**.
[11] Abderhalden and Voitinovici, *ibid.*, **52**, p. 371.

In the tabulation on page 107 we give the cleavage products of certain vegetable proteids; and in certain cases when the analytical results by two investigators differ somewhat, we will give the results side by side. The edestin originated from the hemp-seed, the legumin from the pea, the hordein from barley, the gliadin from wheat and the zein from corn.

Coagulated Proteins. Proteins may be converted into the coagulated condition by different means: by heating, by the action of alcohol, especially in the presence of neutral salts, by chloroform, ether, and metallic salts, and by the prolonged shaking of their solutions (Ramsden[1]), and in certain cases, as in the conversion of fibrinogen into fibrin (Chapter VI), by the action of an enzyme. The nature of the processes which take place during coagulation is unknown. The coagulated albuminous bodies are insoluble in water, in neutral salt solutions, and dilute acids or alkalies, at normal temperature. They are dissolved and converted into albuminates by the action of dilute acids or alkalies, especially on heating.

[1] Arch. f. (Anat. u.) Physiol., 1894.

	Edestin.[1]	Legumin.[3]	Hordein.		Gliadin.		Zein.	
			a^4	b^5	a^6	b^7	a^8	b^9
Glycocoll	3.8	0.38	0.0	....	0.02	0.9	0.0	....
Alanine	3.6	2.08	0.43	1.34	2.0	2.66	2.23	....
Valine	+	1.0[2]	0.13	1.40	0.21	0.33	0.29	....
Leucine	20.9	8.0	5.67	7.0	5.61	6.00	18.60	....
Serine	0.33	0.53	....	0.10	0.13	0.12	0.57	....
Aspartic acid	4.5	5.3	....	1.32	0.5	1.24	1.41	....
Glutamic acid	6.3	13.8	36.35	41.32	37.33	31.5	18.28	....
Cystine	0.25	....	....	....	0.45	....	....	....
Phenylalanine	2.4	3.75	5.03	5.48	2.35	2.6	4.87	....
Tyrosine	2.1	1.55	1.67	4.0	1.20	2.37	3.55	10.10
Proline	1.7	3.22	13.73	5.88	7.06	2.4	6.53	....
Oxyproline	2.0	....	+	....	....	....	....	....
Tryptophane	+	+	....	....	+	1.0	0.0	....
Histidine	1.1	2.42	1.28	0.51	0.61	1.7	0.43	....
Arginine	11.7	10.12	2.16	3.14	3.16	3.4	1.16	....
Lysine	1.0	4.29	0.0	0.0	0.0	0.0	0.0	....
Ammonia	....	1.49	4.87	4.34	5.11	....	3.61	....

[1] Abderhalden, Zeitschr. f. physiol. Chem., **37** and **40**.

[2] Abderhalden and Babkin, *ibid.*, **47**.

[3] Osborne and Clapp, Journ. of Biol. Chem., **3**.

[4] Osborne and Clapp, Amer. Journ. of Physiol., **19**.

[5] Kleinschmitt, Zeitschr. f. physiol. Chem., **54**.

[6] Osborne and Clapp, Amer. Journ. of Physiol., **17**.

[7] Abderhalden and Samuely, Zeitschr. f. physiol. Chem., **44**, and Abderhalden, Lehrbuch d. physiol. Chem., 1909.

[8] Osborne and Clapp, Amer. Journ. of Physiol., **19**.

[9] Kutscher, Zeitschr. f. physiol. Chem., **38**.

Coagulated proteins also seem to occur in animal tissues. We find, at least in many organs such as the liver and other glands, proteins which are not soluble in water, dilute salt solutions, or very dilute alkalies, and only dissolve after being modified by strong alkalies.

Histones are basic proteins which stand to a certain extent between the strongly basic protamines (see below) and the true proteins. Their content of nitrogen varies between 16.5 and 19.8 per cent, and in certain instances is not higher than in other proteins, especially vegetable proteins. According to Kossel and Kutscher and Lawrow they are, on the contrary, richer in basic nitrogen, and especially yield more arginine than other proteins. Kossel first isolated a peculiar protein substance from the red corpuscles of goose blood which was precipitated by ammonia, and because of its similarity in certain regards to the peptones (in the old sense) he called it histone. At the present time a number of very different bodies are described as histones, such as those obtained from nucleohistone (Lilienfeld), from hæmoglobin (globin according to Schulz), from mackerel spermatozoa (scombron according to Bang), from the codfish (gadushistone according to Kossel and Kutscher),

from the burbot, (lotahistone, EHRSTRÖM), and from the sea-urchin (arbacin, MATHEWS)[1].

Sulphur has been found in those histones in which it has been tested for, but they do not, at least not all, give the lead-blackening test with alkali and lead acetate. They give the biuret test, but as a rule only a faint MILLON'S reaction. The goose-blood histone first studied by KOSSEL gives the three following reactions: The neutral salt-free solution first, does not coagulate on boiling; second, gives a precipitate with ammonia which is insoluble in an excess of the precipitant; third, gives a precipitate with nitric acid which disappears on heating and reappears on cooling.

The different histones behave differently in these three reactions, and hence they are not specific. On the other hand, all histones seem to be precipitated from neutral solution by alkaloid reagents, and they also produce precipitates in protein solutions. These two reactions are likewise not specific for the histones, as the protamines have a similar behavior. The histones differ from the protamines by having a much lower content of basic nitrogen, and also probably by always containing sulphur. True proteins, as OSBORNE'S[2] edestan, also give these two reactions; therefore it is impossible by qualitative tests alone to identify a substance as a histone with positiveness. The large content of basic nitrogen and of arginine is not a sure point of difference between histones and other bodies. Histone yields little more than 40 per cent basic nitrogen, while a heteroproteose yields about the same, namely, 39 per cent. Histone yields 14–15.5 per cent arginine (gadushistone), and the lotahistone only 12 per cent. The vegetable proteid excelsin is rich in arginine, namely, 14.14 per cent (OSBORNE and CLAPP[3]). The characteristics of the histones according to KOSSEL are the above-given reactions and the high amount of hexone bases, especially arginine. The arginine nitrogen amounts to about 25 per cent of the total nitrogen, the lysine N = 7–8.5 per cent and the histidine N = 1.8–4.5 per cent. No proteids, with the exception of certain protamines, are known for the present, which contain as much arginine and lysine as the histones. On hydrolytic cleavage the histones, like other proteins, but unlike the protamines, yield a large number of monamino-acids. ABDERHALDEN and RONA[4] obtained from thymus histone the following: leucine 11.8,

[1] Kossel, Zeitschr. f. physiol. Chem., 8, and Sitzungbers. der Gesellsch. zur Beförd. d. ges. Naturwiss. zu Marburg, 1897; Kossel and Kutscher, *ibid.*, 1900, and Zeitschr. f. physiol. Chem., **31**; Lawrow, *ibid.*, 28, and Ber. d. d. chem. Gesellsch., **34**; Lilienfeld, Zeitschr. f. physiol. Chem., **18**; Schulz, *ibid.*, **24**; Bang, *ibid.*, **27**; Ehrström, *ibid.*, **32**; Mathews, *ibid.*, **23**.

[2] Zeitschr. f. physiol. Chem., **33**.

[3] Amer. Journ. of Physiol., **19**.

[4] Zeitschr. f. physiol. Chem., 41.

alanine 3.46, glycocoll 0.50, proline 1.46, phenylalanine 2.20, tyrosine 5.20, and glutamic acid 0.53 per cent.

On pepsin digestion the histones, according to KOSSEL and PRINGLE [1] yield so-called histone-peptone, which also contains 25 per cent of the total nitrogen as arginine nitrogen. This histone-peptone differs from the protamines in not giving a precipitate with proteid in neutral or ammoniacal solution, but is precipitated in neutral reactions by sodium picrate. This property is used in its isolation.

According to KOSSEL the histones are probably intermediate bodies between the protamines and protein bodies on the demolition of the latter, and if this be true, then it is not to be expected that a sharp differentiation exists between histone and proteid, and for this reason it is hardly possible for the present to give a precise definition for the histones.

The **parahistone** found by FLEROFF in the thymus gland yields so little basic nitrogen that it probably does not belong to the histone group (KOSSEL and KUTSCHER [2]).

Protamines. In close relation to the proteins stands a group of substances, the protamines, discovered by MIESHER, which are designated by KOSSEL as the simplest proteins or as the nucleus of the protein bodies. Thus far they have been found only in combination with nucleic acids in fish spermatozoa,[3] and the investigations of KOSSEL and WEISS [4] have shown that the material from which the protamines are formed, at least in the salmon, is the muscle proteid. The question has been raised whether the protamines are true proteids or not, and whether it would not be more correct to consider them as cleavage products of proteid, or as fractious thereof. According to the generally accepted view we will treat them as true proteids.

Protamine was discovered by MIESCHER [5] in salmon spermatozoa.

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[1] Zeitschr. f. physiol. Chem., **49**.

[2] Fleroff, Zeitschr. f. physiol. Chem., **28**; Kossel and Kutscher, l.c.

[3] Nelson, Arch. f. exp. Path. u. Pharm., **59**, has recently shown that the body called by him thymamin and prepared from the thymus glands, is a protamine, still he has not given sufficient evidence of the protamine nature of the substance.

[4] Zeitschr. f. physiol. Chem., **52**.

[5] In regard to protamines, see Miescher, Histochemische und Physiologische Arbeiten, Leipzig, 1897; Piccard, Ber. d. deutsch. chem. Gesellsch., **7**; Schmiedeberg, Arch. f. exp. Path. u. Pharm., **37**; Kossel, Zeitschr. f. physiol. Chem., **22** (Ueber die basischen Stoffe des Zellkerns), **25**, 165 and 190, **26**, **40**, and **44**, and Sitzungsber. der Gesellsch. zur Beförd. der ges. Naturwiss. zu Marburg, 1897; Berl. klin. Wochenschr., 1904; Kossel and Mathews, Zeitschr. f. physiol. Chem., **23** and **25**; Kossel and Kutscher, *ibid.*, **31**; Goto, *ibid.*, **37**; Kurajeff, *ibid.*, **32**; Morkowin, *ibid.*, **28**; Kossel and Dakin, *ibid.*, **40**, **41**, and **44**; Malenück, *ibid.*, **57**; Nelson, Arch. f. exp. Path. u. Pharm., **59**.

Later KOSSEL isolated and studied similar bases from the spermatozoa of herring, sturgeon, mackerel, and other fishes. As all these bases are not identical, KOSSEL uses the name protamines to designate the group, and calls the individual protamines according to their origin *salmine, clupeine, scombine, sturine, cyprinine, cyclopterine,* etc.

They differ essentially from the proteins by the fact that they yield chiefly diamino-acids (always abundant arginine) as cleavage products, and only a small amount of monamino-acids. They are strongly basic substances rich in nitrogen (about 30 per cent or more) and have high moleculer weight.

The percentage composition of these bodies has not been satisfactorily determined. As probable formulæ we have for salmine $C_{32}H_{54}N_{18}O_4$ (MIESCHER, SCHMIDEBERG, NELSON), or $C_{30}H_{57}H_{17}O_6$ (KOSSEL and GOTO), for clupeine $C_{30}H_{62}N_{14}O_9$, and for sturine $C_{36}H_{69}H_{19}O_7$ (KOSSEL) or $C_{34}H_{71}N_{17}O_9$ (GOTO), or according to MALENÜCK $C_{27}H_{55}H_{13}P_7$ for sturine from Accipenser Guldenstadtii. On boiling with dilute mineral acids, as also by tryptic digestion, the protamines first yield peptone-like substances called *protones*, from which simple products are derived on further cleavage. All protamines yield arginine, the four protamines salmine, clupeine, cyclopterine, and sturine, yielding 87.4, 82.2, 62.5, and 58.2 per cent respectively. In the three protamines salmine, clupeine and scombrine the arginine nitrogen, according to KOSSEL and PRINGLE,[1] amounts to about 89 per cent of the total nitrogen. Sturine yields besides this the two hexone bases lysine, 12 per cent, and histidine, 12.9 per cent. Histidine has not been found in any other protamine. The carp protamine, cyprinine, occurs in two different modifications, namely, α- and β-cyprinine. The α-cyprinine yields only little arginine, 4.9 per cent, but the lysine content is pronounced, 28.8 per cent. Of the total nitrogen 30.3 per cent exists as lysine. KOSSEL and DAKIN have obtained from salmine the following cleavage products, namely, arginine 87.4, serine 7.8, aminovaleric acid 4.3, and α-pyrrolidine-carboxylic acid 11 per cent, and according to them the salmine contains about 10 mol. arginine, 2 mol. serine, 1 mol. aminovaleric acid, and 2 mol. proline. Scombrine contains only arginine, alanine, and proline. KOSSEL believes that diarginide or polyarginide groups also occur in the protamines, and in clupeine we can accept the presence of diarginylalanine, diarginylserine, diarginylproline, and diarginylvaline (KOSSEL and PRINGLE).

The following summary according to KOSSEL[2] gives a view of the cleavage products of the protamines thus far investigated:

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[1] Zeitschr. f. physiol. Chem., 49.

[2] *Ibid.*, 44.

	Scombrine.	Salmine.	Clupeine.	Sturine.	Cyclopterine.	α-Cyprinine.	β-Cyprinine.
Alanine	+	0	+	+	?	?	?
Serine	0	+	+	0	?	?	?
Aminovaleric acid	0	+	+	0	?	+	+
Leucine	0	0	0	+	?	?	?
Arginine	+	+	+	+	+	+	+
Lysine	0	0	0	+	0	+	+
Histidine	0	0	0	+	0	0	0
Proline	+	+	+	0	?	?	?
Tyrosine	0	0	0	0	+	0	0
Tryptophane	0	0	0	0	+	0	0

Solutions of these bases in water are alkaline and have the property of giving precipitates with ammoniacal solutions of proteins or primary proteoses, but the researches of HUNTER [1] show that these precipitates are not histones, as generally considered. The salts with mineral acids are soluble in water, but insoluble in alcohol and ether. They are more or less readily precipitated by neutral salts (NaCl). Among the salts of the protamines, the sulphate, picrate, and the double-platinum chloride are the most important, and are used in the preparation of the protamines. The protamines are, like the proteins, levogyrate. They give the biuret test beautifully, but with the exception of cyclopterine and β-cyprinine do not give MILLON'S reaction. The protamine salts are precipitated in neutral or even faintly alkaline solutions by phosphotungstic acid, picric acid, chromic acid, and alkali ferrocyanides.

The protamines are prepared, according to KOSSEL, by extracting the heads of the spermatozoa, which have previously been extracted with alcohol and ether, with dilute sulphuric acid (1–2 per cent), filtering, and precipitating with 4 vols. of alcohol. The sulphate may be purified by repeated solution in water and precipitation with alcohol, and if necessary, conversion into the picrate. For more details see the works of KOSSEL and MALENÜCK. The double-platinum salt is best suited for analysis and can be obtained, according to GOTO, by precipitating the methyl-alcohol solution of the protamine hydrochloride with platinum chloride. MIESCHER also precipitates the base as a double-platinum salt.

B. Albuminoids or Albumoids.

Under this name we collect into a special group all those protein bodies which cannot be placed in either of the other groups. Most and best studied of the bodies belonging to this group are important constituents of the animal skeleton or the cutaneous structure. Some are hardened secretions, and all occur as a rule in an insoluble state in the organism, and they are distinguished in most cases by a pronounced

[1] Zeitschr. f. physiol. Chem., 53.

resistance to reagents which dissolve proteins, or to chemical reagents in general, and it is due to these external properties that they are put in a special group. From a purely chemical standpoint there is no reason why they should be separated from the true proteids in a special group. Most of the bodies belonging to the albuminoids have been given on page 91.

The Keratins. Keratin is the chief constituent of the horny structure of the epidermis, of hair, wool, of the nails, hoofs, horns, feathers, of tortoise shell, etc., etc. Keratin is also found as neurokeratin (KÜHNE) in the brain and nerves. The shell membrane of the hen's egg seems also to consist of keratin, and according to NEUMEISTER[1] the organic matrix of the eggshells of various vertebrate animals belongs in most cases to the keratin group.

It seems that there exist a number of keratins, and these form a special group of bodies. This fact, together with the difficulty in isolating the keratin from the tissues in a pure condition without a partial decomposition, is sufficient explanation for the variation in the elementary composition given below. As examples the analyses of a few tissues rich in keratin and of keratins are given:[2]

	C	H	N	S	O	
Human hair . . .	50.65	6.36	17.14	5.00	20.95	(v. LAAR)
Nail	51.00	6.94	17.51	2.80	21.75	(MULDER)
Neurokeratin . .	56.11–58.45	7.26–8.02	11.46–14.32	1.63–2.24	. . . . .	(KÜHNE)
Neurokeratin. . .	56.61	7.45	14.17	2.27	. . . . .	(ARGIRIS)
Horn (average).	50.86	6.94	. . . . .	3.20	. . . . .	(HORBACZEWSKI)
Tortoise shell . .	54.89	6.56	16.77	2.22	19.56	(MULDER)
Shell membrane	49.78	6.64	16.43	4.25	22.90	(LINDVALL)
Egg membrane. (Scyllium)	53.92	7.33	15.08	1.44	. . . . .	(PREGL)

MOHR[3] has determined the quantity of sulphur in various keratin substances. Sulphur is in great part in loose combination, and it is chiefly removed by the action of alkalies (as sulphides), or indeed in part by boiling with water. Combs of lead after long usage become black, and this is due to the action of the sulphur of the hair. On heating keratin with water in sealed tubes to a temperature of 150° C. or higher, it dissolves with the elimination of sulphuretted hydrogen or mercaptan (BAUER), and the solution contains proteose-like substances (KRUKEN-

[1] Kühne and Ewald, Verh. d. naturhistor.-med. Vereins zu Heidelberg (N. F.), **1**; also Kühne and Chittenden, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **31**.

[2] v. Laar, Annal. d. Chem. u. Pharm., **45**; Mulder, Versuch einer allgem. physiol. Chem., Braunschweig, 1844–51; Kühne, Zeitschr. f. Biologie, **26**; Horbaczewski, see Drechsel in Ladenburg's Handwörterbuch. d Chem., **3**; Lindvall, Maly's Jahresbericht, 1881; Argiris, Zeitschr. f. physiol. Chem., **54**; Pregl., *ibid.*, **56**.

[3] Zeitschr. f. physiol. Chem., **20**.

BERG) called *atmidkeratin* and *atmidkeratose* by BAUER.[1] Keratin is dissolved by alkalies, especially on warming, producing besides alkali sulphides also proteose substances.

Besides the well-known cleavage products such as leucine, tyrosine, aspartic acid, glutamic acid, arginine, and lysine, FISCHER and DÖRPINGHAUS,[2] have recently found glycocoll, alanine, α-aminovaleric acid, proline, serine, phenylalanine, and pyrrolidone-carboxylic acid (secondary from glutamic acid) among the cleavage products of horn substances. EMMERLING claims to have found cystine as a sulphurized cleavage product, but K. MÖRNER was the first to positively prove the abundant occurrence of cystine in the cleavage products. MÖRNER obtained from ox-horn, human hair, and the shell-membrane of the hen's egg 6.8, 13.92, and 7.62 per cent cystine calculated on the basis of the dry substance. BUCHTALA [3] obtained the following amounts of cystine from the respective keratin formations, namely, 12.98–14.53 per cent from human hair, 5.15 per cent from nails, 7.98 per cent from horsehair, 3.20 per cent from horse hoofs, 7.27 per cent from ox hair, 5.37 per cent from ox hoofs, 7.22 from pig bristles and 2.17 per cent from pig hoofs. From the amount of sulphur split off by alkali, MÖRNER concludes that, at least in ox horn and human hair, all the sulphur exists as cystine. GALIMARD [4] was able to get only a qualitative test for cystine in the keratin of the adder eggs. SUTER, MÖRNER, and FRIEDMANN [5] have obtained α-thiolactic acid as a hydrolytic cleavage product of the keratin substances. The last-mentioned investigator was also able to detect thioglycolic acid in the cleavage products of wool.

The shell membrane of the hen's egg and the eggshells of amphibians and certain fishes are, as above mentioned, ordinarily classified as keratins. These bodies among themselves, as well as on comparison with other keratins, show a marked difference in properties, this being very evident from the tabulation on page 114.

The large quantity of cystine in the keratins is considered as especially characteristic, and they differ in this regard from the other proteins. The shell membrane of the hen's egg behaves like a keratin in regard to the large amount of cystine contained, but differs essentially by the absence

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[1] Krukenberg, Untersuch. über d. chem. Bau d. Eiweisskörper, Sitzunsber. d. Jenaischen Gesellsch. f. Med. u. Naturwissensch., 1886; Bauer, Zeitschr. f. physiol. Chem., **35**.

[2] Zeitschr. f. physiol. Chem., **36**, which contains also the older literature.

[3] Mörner, *ibid.*, **34** and **42**; Emmerling, Ref. in Chemiker Zeitung, 1894; Buchtala, Zeitschr. f. physiol. Chem., **52**.

[4] Chem. Centralbl. II, 1905.

[5] Suter, Zeitschr. f. physiol. Chem., **20**; Mörner, *ibid.*, **42**; Friedmann, Hofmeister's Beiträge, **2**.

of tyrosine. It is remarkable that the egg membrane of the Selachii, which biologically is analogous with ovokeratin, differs from the typical keratins by the absence of cystine, while it contains, on the contrary, large amounts of tyrosine. The typical keratins differ among themselves in regard to composition, thus the keratin from the sheep hoofs contains 2 per cent phenylalanine while this amino-acid is absent in the keratin of hair and feathers. It is difficult to say whether or not this is due to a difference in the purity of the bodies or not. The keratins as thus far investigated do not chemically form a sufficient characteristic group.

	Keratin from Horse-hair.[1]	Keratin from Sheep Wool.[4]	Keratin from Goose Feathers[5]	Keratin from Sheep Horn.[4]	Shell Membrane of the Hen's egg.[6]	Egg Membrane of *Scyllium stellare*.[8]	Egg Membrane of *Testudo graeca*.[9]
Glycocoll	4.7	0.58	2.6	0.45	3.9	2.6	+
Alanine	1.5	4.40	1.8	1.6	3.5	3.2	+
Valine	0.9	2.80	0.5	4.5	1.1	—	—
Leucine	7.1	11.5	8.0	15.3	7.4	5.8	+
Serine	0.6	0.1	0.4	1.1	—	—	—
Aspartic acid	0.3	2.3	1.1	2.5	1.1	2.3	1.2
Glutamic acid [10]	3.7	12.9	2.3	17.2	8.1	7.2	2.9
Cystine	7.98[2]	7.3	—	7.5	7.62[7]	?	—
Phenylalanine	0.0	—	0.0	1.9	—	3.3	+
Tyrosine	3.2	2.9	3.6	3.6	0.0	10.6	—
Proline	3.4	4.4	3.5	3.7	4.0	4.4	11.8
Histidine	0.61[3]	—	—	—	—	1.7	—
Arginine	4.45[3]	—	—	2.7	—	3.2	—
Lysine	1.12[3]	—	—	0.2	—	3.7	—

[1] Abderhalden and Wells, Zeitschr. f. physiol. Chem., **46**.

[2] Buchtala, *ibid.*, **52**.

[3] Argiris, *ibid.*, **54**.

[4] Abderhalden and Voitinovici, *ibid.*, **52**.

[5] Abderhalden and Le Count, *ibid.*, **46**.

[6] Abderhalden and Ebstein, *ibid.*, **48**.

[7] Körner, *ibid.*, **34** and **42**.

[8] Pregl, *ibid.*, **56**.

[9] Abderhalden and Strauss, *ibid.*, **48**.

[10] Abderhalden and Fuchs, Zeitschr. f. physiol. Chem., **57**, have recently shown that the same variety of keratin, on ageing of the horn structure, becomes somewhat poorer in glutamic acid.

Bodies occur in the animal kingdom which form to a certain extent intermediate substances between coagulated protein and keratin. C. Th. Mörner[1] has detected such a body (*albumoid*) in the tracheal cartilage which forms a net-like trabecular tissue. This substance appears to be related to the keratins on account of its solubilities and the quan-

[1] See Maly's Jahresber., **18**.

tity of the sulphur (lead-blackening) it contains, while according to its solubility in gastric juice it must stand close to the proteins. Another substance, nearly like keratin, is the horny layer in the gizzard of birds. According to J. HEDENIUS this substance is insoluble in gastric or pancreatic juice, and acts quite like keratin. According to K. B. HOFMANN and PREGL,[1] who call this substance *koilin*, it does not yield any cystine on hydrolysis, or at least not a determinable quantity, and differs from the keratins in this and other regards.

Keratin is amorphous or takes the form of the tissues from which it was prepared. It is insoluble in water, alcohol, or ether. On heating with water to 150–200° C. it dissolves. It also dissolves gradually in caustic alkalies, especially on heating. It is not dissolved by artificial gastric juice or by trypsin solutions. Keratin gives the xanthoproteic reaction, as well as the reaction with MILLON'S reagent, although the latter is not always typical.

In the preparation of keratin a finely divided horny structure is treated first with boiling water, then consecutively with diluted acid, pepsin-hydrochloric acid, and alkaline trypsin solution, and, lastly, with water, alcohol, and ether.

Elastin occurs in the connective tissue of higher animals, sometimes in such large quantities that it forms a special tissue. It occurs most abundantly in the cervical ligament (ligamentum nuchæ).

Elastin used to be generally considered as a sulphur-free substance. According to the investigations of CHITTENDEN and HART, it is a question whether or not elastin contains sulphur, as it may have been removed by the action of the alkali in its preparation. H. SCHWARZ has been able to prepare an elastin containing sulphur from the aorta by another method, and this sulphur can be removed by the action of alkalies, without changing the properties of the elastin; and recently ZOJA, HEDIN, BERGH, and RICHARDS and GIES[2] have found that elastin contains sulphur. The most trustworthy analyses of elastin from the cervical ligament (Nos. 1 and 2) and from the aorta (No. 3) have given the following results, which compare well with each other:

	C	H	N	S	O	
1.	54.32	6.99	16.75	....	21.94	(HORBACZEWSKI[3])
2.	54.24	7.27	16.70	....	21.79	(CHITTEDDEN and HART)
3.	53.95	7.03	16.67	0.38	.....	(H. SCHWARZ)

[1] Hedenius, Skand. Arch. f. Physiol., **3**; Hofmann and Pregl, Zeitschr. f. physiol., Chem., **52**.

[2] Chittenden and Hart, Zeitschr. f. Biologie, **25**; Schwarz, Zeitschr. f. physiol. Chem., **18**; Zoja, *ibid.*, **23**; Bergh, *ibid.*, **25**; Hedin, *ibid.*; Richards and Gies, Amer. Journ of Physiol., **7**.

[3] Zeitschr. f. physiol. Chem., **6**.

Zoja found 0.276 per cent sulphur and 16.96 per cent nitrogen in elastin. Hedin and Bergh found different quantities of nitrogen in aorta-elastin, depending upon whether Horbaczewski's or Schwarz's method was used in its preparation. In the first case they found 15.44 per cent nitrogen and 0.55 per cent sulphur, and in the other 14.67 per cent nitrogen and 0.66 per cent sulphur. Richards and Gies found 0.14 per cent sulphur and 16.87 per cent nitrogen in elastin.

The quantity of hydrolytic cleavage products are given in the table on page 124. It is sufficient to here call attention to the fact that no aspartic acid and only very little glutamic acid have been found. The hexone bases have been obtained, but only in very small amounts, so that the basic nitrogen represents only 3.34 per cent of the total nitrogen (Richards and Gies). This fact and the very low sulphur content make it questionable whether the elastin is a unit body.

Indol and skatol have not been found on the putrefaction of elastin,[1] but Schwarz, on the contrary, obtained indol, skatol, benzene, and phenols on fusing aorta-elastin with caustic potash. On heating with water in closed vessels, on boiling with dilute acids, or by the action of proteolytic enzymes, the elastin dissolves and splits into two chief products, called by Horbaczewski *hemielastin* and *elastinpeptone*. According to Chittenden and Hart, these products correspond to two proteoses designated by them *protoelastose* and *deuteroelastose*. The first is soluble in cold water and separates out on heating, and its solution is precipitated by mineral acid as well as by acetic acid and potassium ferrocyanide. The aqueous solution of the other does not become cloudy on heating, and is not precipitated by the above-mentioned reagents. According to Richards and Gies, elastoses, especially protoelastoses, and true peptones are formed, the latter only to a slight extent.

Pure elastin when dry is a yellowish-white powder; in the moist state it appears like yellowish-white threads or membranes. It is insoluble in water, alcohol, or ether, and shows a resistance toward the action of chemical reagents. It is not dissolved by strong caustic alkalies at the ordinary temperature and only slowly at the boiling temperature. It is very slowly attacked by cold concentrated sulphuric acid, but it is relatively easily dissolved on warming with strong nitric acid. Elastins of different origins act differently with cold concentrated hydrochloric acid; for instance, elastin from the aorta dissolves readily therein, while elastin from the ligamentum nuchæ, at least from old animals, dissolves with difficulty. Elastin is more readily dissolved by warm concentrated hydrochloric acid. It responds to the xanthoproteic reaction and to that with Millon's reagent.

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[1] See Wälchli, Journ. f. präkt. Chem. (N. F.), **17**.

On account of its great resistance to chemical reagents, elastin may be prepared (best from the ligamentum nuchæ) in the following way: First boil with water, then with 1 per cent caustic potash, then again with water, and lastly with acetic acid. The residue is treated with cold 5 per cent hydrochloric acid for twenty-four hours, carefully washed with water, boiled again with water, and then treated with alcohol and ether.

In regard to the methods used by SCHWARZ and by RICHARDS and GIES, which are somewhat different, we refer to the original publications.

Collagen, or gelatin-forming substance, occurs very extensively in vertebrates. The flesh of cephalopods is also said to contain collagen.[1] Collagen is the chief constituent of the fibrils of the connective tissue and (as ossein) of the organic substances of the bony structure. It also occurs in the cartilaginous tissues as chief constituent; but it is here mixed with other substances, producing what was formerly called chondrigen. Collagen from different tissues has not quite the same composition, and probably there are several varieties of collagen.

By continued boiling with water (more easily in the presence of a little acid) collagen is converted into gelatin. HOFMEISTER[2] found that gelatin on being heated to 130° C. is again transformed into collagen; and this last may be considered as the anhydride of gelatin. Collagen and gelatin have about the same composition.[3]

	C	H	N	S	O	
Collagen	50.75	6.47	17.86	24.92		(HOFMEISTER)
Gelatin (commerical)	49.38	6.80	17.97	0.7	25.13	(CHITTENDEN)
Gelatin from tendons	50.11	6.56	17.81	0.26	25.26	(VAN NAME)
Gelatin from ligaments	50.49	6.71	17.90	0.57	24.33	(RICHARDS and GIES)
Fish glue (isinglass)	48.69	6.76	17.68	....	.....	(FAUST)

Gelatins of different origin show a somewhat variable composition, which seems to indicate the occurrence of different collagens. It is difficult to say whether the variable content of sulphur is due to a contamination with a substance rich in sulphur or to a splitting off of loosely combined sulphur during the purification. C. MÖRNER[4] has prepared a typical gelatin containing only 0.2 per cent of sulphur by a method which eliminated any possible changes due to reagents.

SADIKOFF[5] has prepared gelatins by various methods from tendons and from cartilage. Those from tendons, some of which were prepared after previous tryptic digestion, some after treatment with 0.25 per cent caustic potash,

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[1] Hoppe-Seyler, Physiol. Chem., p. 97.

[2] Zeitschr. f. physiol. Chem., 2.

[3] Hofmeiser, l. c.; Chittenden and Solley, Journ. of Physiol., **12**; van Name, Journ. of Exper. Med., **2**; Richards and Gies, Amer. Journ. of Physiol., **8**; Faust, Arch. f. exp. Path. u. Pharm., **41**.

[4] Zeitschr. f. physiol. Chem., **28**.

[5] *Ibid.*, **39** and **41**.

and some after treatment with sodium hydroxide and then carbonate, showed somewhat different physical properties among each other, but had about the same elementary composition, with 0.30–0.526 per cent sulphur. SADIKOFF seems to think that the gelatins prepared up to this time were perhaps not unit bodies but were possibly mixtures. The bodies prepared by SADIKOFF from cartilage he calls *gluteins*, because they were essentially different from the other gelatins or glutins. They were poorer in carbon and nitrogen, 17.17 to 17.87 per cent, but somehwat richer in sulphur, 0.53–0.712 per cent, than the tendon glutin. The gluteins differ also from the glutins in that on boiling with a mineral acid they have a faint reducing action, and also in that they give a color reaction with phloroglucin-hydrochloric acid. The glutins differ from the gluteins by a different behavior with certain salts.

The decomposition products of the collagens are the same as those of the gelatins and will be found in the table on page 124. Of special mention is the fact the gelatin contains no tyrosine but does yield considerable glycocoll. This latter substance has, because of its sweet taste, been called gelatin sugar. SKRAUP[1] has obtained on the hydrolytic cleavage of gelatin a crystalline acid having the formula $C_{12}H_{25}N_5O_{10}$, which he calls *glutinic acid*. Gelatin yields considerable basic nitrogen, according to HAUSMANN,[2] 35.83 per cent of the total nitrogen. DRECHSEL and FISCHER found lysine; HEDIN, KOSSEL and KUTSCHER[3] found arginine also, which amounted to 9.3 per cent (KOSSEL and KUTSCHER). On putrefaction gelatin gives neither tyrosine, indol, nor skatol. According to SELTRENNY[4] it yields phenylpropionic acid and phenylacetic acid. The aromatic group in gelatin is therefore, as directly shown by FISCHER and also by SPIRO,[5] represented by phenylalanine.

On the oxidation of gelatin with potassium permanganate, SEEMANN obtained besides volatile fatty acids (formic, acetic, butyric acids), benzoic acid, oxalic acid, succinic acid, oxaluramide and probably also oxaluric acid. ZICKGRAF[6] produced guanidine from the arginine.

Collagen is insoluble in water, salt solutions, and dilute acids and alkalies, but it swells up in dilute acids. By continued boiling with water it is converted into gelatin. Various collagens are converted into gelatin with varying readiness; the formation of gelatin occurs also from difficultly soluble collagens by continuous boiling with water.

Collagen is dissolved by the gastric juice and also by the pancreatic juice (trypsin solution) when it has previously been treated with acid

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[1] Monatshefte f. Chem., **26**.

[2] Zeitschr. f. physiol. Chem., **27**.

[3] Drechsel, Arch. f. Anat. u. Physiol., 1891; Hedin, Zeitschr. f. physiol. Chem., **21**; Kossel and Kutscher, *ibid.*, **31**.

[4] Monatshefte f. Chem., **10**.

[5] Fischer, Levene and Aders, Zeitschr. f. physiol. Chem., **35**; Spiro, Hofmeister's Beiträge, **1**.

[6] Seemann, Zeitschr. f. physiol. Chem., **44**; Zickgraf, *ibid.*, **41**.

or heated with water above 70° C.[1] By the action of ferrous sulphate, corrosive sublimate, or tannic acid, collagen shrinks greatly. Collagen treated by these bodies does not putrefy, and tannic acid is therefore of great importance in the preparation of leather.

Gelatin or glutin is colorless, amorphous, and transparent in thin layers. It swells in cold water without dissolving. It dissolves in warm water, forming a sticky liquid, which solidifies on cooling when sufficiently concentrated. As PAULI and RONA[2] have shown, various bodies may have a different influence upon the gelatinization-point of a gelatin solution; thus certain substances such as sulphates, citrates, acetates, and glycerin may accelerate, while the chlorides, chlorates, bromides, alcohol, and urea retard, this power.

Gelatin solutions are not precipitated on boiling, or by mineral acids, acetic acid, alum, basic lead acetate, or metallic salts in general. A gelatin solution acidified with acetic acid may be precipitated by potassium ferrocyanide on carefully adding the reagent. Gelatin solutions are precipitated by tannic acid in the presence of salt; by acetic acid and common salt in substance; mercuric chloride in the presence of HCl and NaCl; by metaphosphoric acid and phosphomolybdic acid in the presence of acid; and lastly also by alcohol, especially when neutral salts are present. Gelatin solutions do not diffuse. Gelatin gives the biuret reaction, but not ADAMKIEWICZ'S. It gives MILLON'S reaction and the xanthoproteic reaction so faintly that they probably occur from impurities consisting of proteids. According to C. MÖRNER, pure gelatin gives a beautiful MILLON'S reaction, if not too much reagent is added. In the other case no reaction or only a faint one is obtained.

By continued boiling with water gelatin is converted into a non-gelatinizing modification called β-glutin by NASSE. According to NASSE and KRÜGER the specific rotatory power is hereby reduced from −167.5° to about −136°.[3] On prolonged boiling with water, especially in the presence of dilute acids, also in the gastric or tryptic digestion, the gelatin is transformed into gelatin proteoses, so-called *gelatoses* and *gelatin peptones*, which diffuse more or less readily.

According to HOFMEISTER two new substances, *semiglutin* and *hemicollin*, are formed. The former is insoluble in alcohol of 70–80 per cent and is precipitated by platinum chloride. The latter, which is not precipitated by platinum chloride, is soluble in alcohol. CHITTENDEN and SOLLEY[4] have obtained in the peptic and tryptic digestion a *proto-* and

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[1] Kühne and Ewald, Verh. d. Naturhist. Med. Vereins in Heidelberg, 1877, **1**.

[2] Hofmeister's Beiträge, **2**.

[3] Nasse and Krüger, Maly's Jahresber., **19**, p. 29. In regard to the rotation of β-glutin, see Framm, Pflüger's Arch., **68**.

[4] Hofmeister, l. c.; Chittenden and Solley, l. c.

a *deutero-gelatose*, besides a true peptone. The elementary composition of these gelatoses does not essentially differ from that of the gelatin.

According to LEVENE the proto- as well as the deuterogelatoses yield a larger amount of glycocoll than the gelatin itself. On prolonged tryptic digestion a further demolition takes place, so that the peptone yields only about the same amount of glycocoll as the gelatin. Some leucine and, as it appears, also some glutamic acid and phenylalanine are split off. Quite a considerable splitting off of NH_3 also takes place (LEVENE and STOOKEY).[1]

PAAL[2] has prepared gelatin-peptone hydrochlorides from gelatin by the action of dilute hydrochloric acid. These salts are partly soluble in ethyl and methyl alcohol, and partly insoluble therein. The peptones obtained from these salts contain less carbon and more hydrogen than the gelatin from which they originated, showing that hydration has taken place. The molecular weight of the gelatin peptone as determined by PAAL, by RAOULT'S cryoscopic method, was 200 to 352, while that for gelatin was 878 to 950. The gelatin peptones isolated by SIEGFRIED and his pupils SCHEERMESSER and KRÜGER and which will be discussed below, are of great interest.

Collagen (contaminated with mucoid) may be obtained from bones by extracting them with hydrochloric acid (which dissolves the earthy phosphates) and then carefully washing the acid out with water. It may be obtained from tendons by extracting with lime-water or dilute alkali (which dissolve the proteids and mucin) and then thoroughly washing with water. Gelatin is obtained by boiling collagen with water. The finest commercial gelatin always contains a little proteid, which may be removed by allowing the finely divided gelatin to swell up in water and thoroughly extracting with large quantities of fresh water. Then dissolve in warm water and precipitate with alcohol.

Collagen may also be purified from proteids, as suggested by VAN NAME, by digesting with an alkaline trypsin solution or by extracting the gelatin for many days with 1–5 p. m. caustic potash, as suggested by C. MÖRNER. The typical properties of gelatin are not changed by this.

Chondrin or cartilage gelatin is only a mixture of gelatin with the specific constituents of the cartilage and their transformation products.

Reticulin. The reticular tissues of the lymphatic glands contain a variety of fibers which have also been found by MALL in the spleen, intestinal mucosa, liver, kidneys, and lungs. These fibers consist of a special substance, reticulin, investigated by SIEGFRIED.[3]

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[1] Levene, Zeitschr. f. physiol. Chem., **37**; Levene and Stookey, *ibid.*, **41**.

[2] Ber. d. deutsch. chem. Gesellsch., **25**.

[3] Mall, Abhandl. d. math.-phys. Klasse d. Kgl. sächs. Gesellsch. d. Wiss., 1891; Siegfried, Ueber die chem. Eigensch. der retikulirten Gewebe, Habil.-Schrift, Leipzig, 1892.

Reticulin has the following composition: C 52.88; H 6.97; N 15.63; S 1.88; P 0.34; ash 2.27 per cent. The phosphorus occurs in organic combination. It yields no tyrosine on cleavage with hydrochloric acid. It yields, on the contrary, sulphuretted hydrogen, ammonia, lysine, arginine, and valine. On continued boiling with water, or more readily with dilute alkalies, reticulin is converted into a body which is precipitated by acetic acid, and at the same time phosphorus is split off.

Reticulin is insoluble in water, alcohol, ether, lime-water, sodium carbonate, and dilute mineral acids. It is dissolved, after several weeks, on standing with caustic soda at the ordinary temperature. Pepsin-hydrochloric acid or trypsin does not dissolve it. Reticulin responds to the biuret, xanthoproteic, and ADAMKIEWICZ's reactions, but not to MILLON's reagent.

According to TEBB reticulin is only a somewhat changed, impure collagen but this is disputed by SIEGFRIED.[1]

It may be prepared as follows, according to SIEGFRIED: Digest intestinal mucosa with trypsin and alkali. Wash the residue, extract with ether, and digest again with trypsin and then treat with alcohol and ether. On careful boiling with water the collagen present either as contamination or as a combination with reticulin is removed. The thoroughly boiled residue consists of reticulin.

Ichthylepidin is an organic compound, so called by C. MÖRNER,[2] which occurs with collagen in fish-scales and forms about one-fifth of their organic substance. This compound, with 15.9 per cent nitrogen and 1.1 per cent sulphur, stands on account of its properties rather close to elastin. It is insoluble in cold and hot water, as well as in dilute acids and alkalies at the ordinary temperature. On boiling with these it dissolves. Pepsin-hydrochloric acid, as well as an alkaline trypsin solution, also dissolves it. It responds beautifully with MILLON's reagent, the xanthoproteic reaction, and the biuret test. At least a part of the sulphur is split off by the action of alkali. Ichthylepidin stands very close to elastin in regard to its solubilities; but it differs essentially in composition as it is markedly poorer in glycocoll, but much richer in proline and glutamic acid than elastin (ABDERHALDEN and VOITINOVICI [3]).

As **skeletins,** KRUKENBERG[4] has designated a number of nitrogenized substances which form the skeletal tissue of various classes of invertebrates. These substances are *chitin, spongin, conchiolin, byssus, cornein,* and *crude silk* (*fibroin* and *sericin*). Of these, chitin does not belong to the protein substances, and silk is hardly to be classed as a skeletin. Only those so-called skeletins will be discussed that actually belong to the protein group, and chitin will be discussed in another chapter.

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[1] Tebb, Journ. of Physiol., 27; Siegfried, *ibid.*, 28.

[2] Zeitschr. f. physiol. Chem., 24 and 37. See also Green and Tower, *ibid.*, 35.

[3] Zeitschr. f. physiol. Chem., 52, p. 368.

[4] Grundzüge einer vergl. Physiol. d. thier. Gerüstsubst. Heidelberg, 1885.

The elementary composition of certain of the bodies belonging to this group is as follows:[1]

	C	H	N	S	
Conchiolin (from the shells of pinna) ..	52.70	6.54	16.60	0.85	(Wetzel)
" (from snail eggs)	50.92	6.88	17.86	0.31	(Krukenberg)
Spongin.....	46.50	6.30	16.20	0.50	(Crookewitt)
"	48.75	6.35	16.40	....	(Posselt)
Cornein.....	48.96	5.90	16.81	....	(Krukenberg)
Fibroin.....	48.23	6.27	18.31	....	(Cramer)
"	48.30	6.50	19.20	....	(Vignon)
Sericin.....	44.32	6.18	18.30	....	(Cramer)
"	44.50	6.32	17.14	....	(Bondi)

Spongin forms the chief mass of the ordinary sponge. It dissolves with difficulty in concentrated mineral acids but dissolves with readiness in caustic alkalies. It does not give the Millon reaction or Adamkiewicz's. It gives no gelatin. On hydrolysis spongin yields considerable glycocoll 13.9 per cent, glutamic acid 18.1 per cent, leucine 7.5 per cent, proline 6.3 per cent, lysin 3–4 per cent, and arginine 5.6 per cent.[2] Tyrosine and phenylalanine could not be detected. After Hundeshagen had shown the occurrence of iodine and bromine in organic combination in different sponges and designated the albumoid containing iodine, *iodospongin*, Harnack [3] later isolated from the ordinary sponge, by cleavage with mineral acids, an iodospongin which contained about 9 per cent iodine and 4.5 per cent sulphur. Strauss [4] has obtained *sponginoses* of various kinds from spongin by dilute acids. The heterosponginose contained the greater part of the iodine and sulphur, while the deuterosponginose contained the carbohydrate groups. Iodospongin is considered as a derivative of the heterosponginose. **Conchiolin** is found in the shells of mussels and snails and also in the eggshells of these animals. It yields, according to Wetzel,[5] glycocoll, leucine, and abundance of tyrosine. The quantity of diamino-nitrogen amounts to 8.7 per cent and the amide nitrogen 3.47 per cent (from the shell of pinna). The **Byssus** contains a substance, closely related to conchiolin, which is soluble with difficulty. According to Abderhalden [6] it yields considerable glycocoll and tyrosine and also alanine, aspartic acid and very large amounts of proline.

Cornein is the name given to the substance of the axial system of certain Anthozoa. The substance occurring in the groups of Gorgonia and Antipathes has been called *gorgonin* by C. Mörner [7] and differs from the *pennatulin* of the Pennatulideæ by the latter being readily soluble in pepsin-hydrochloric acid. The cleavage products have not been carefully studied; one of the crystalline products, called *cornicrystalline* by

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[1] Krukenberg, Ber. d. d. chem. Gesellsch., **17** and **18**, and Zeitschr. f. Biologie, **22**; Croockewitt, Annal. d. Chem. u. Pharm., **48**; Posselt, *ibid.*, **45**; Cramer, Journ. f. prakt. Chem., **96**; Vignon, Compt. rend., **115**; Wetzel, Zeitschr. f. physiol. Chem., **29** and Centralbl. f. Physiol., **13**, 113; Bondi, Zeitschr. f. physiol. Chem., **34**.

[2] Abderhalden and Strauss, Zeitschr. f. physiol. Chem., **48**; Kossel and Kutscher, *ibid.*, **31**, 205.

[3] Zeitschr. f. physiol. Chem., **24**; Hundeshagen, Maly's Jahresber., **25**, 394; see also L. Scott, Biochem. Zeitschr., **1**.

[4] Biochem. Centralbl., **3**.

[5] Zeitschr. f. physiol. Chem. **29**, and Centralbl. f. Physiol., **13**, 113.

[6] Zeitschr. f. physiol. Chem., **55**.

[7] Zeitschr. f. physiol. Chem., **51** and **55**.

KRUKENBERG, is nothing but iodine crystals, as shown by MÖRNER. After DRECHSEL [1] found nearly 8 per cent iodine in the dry substance of the axial system of the Gorgonia Cavolini, C. MÖRNER showed that in the Anthozoa in general the organic skeletal substance contains halogens in organic combination. Iodine was found in all varieties, and indeed in amounts from traces up to 7 per cent. Bromine was found, with the exception of two Antipathes, in amounts of 0.25 to 4 per cent, while chlorine, which was never absent, occurred as a few tenths per cent. The halogens occur in the organic skeletal substance as gorgonin and pennatulin.

DRECHSEL obtained leucine, tyrosine, lysine, ammonia and an iodized amino-acid, *iodogorgonic acid*, as cleavage products of gorgonin. This last is identical with the 3–5 di-iodo-tyrosine, $HOI_2C_6H_2.CH_2.CHNH_2$ COOH, synthetically prepared by WHEELER and JAMIESON.[2] On acid cleavage of gorgonin HENZE [3] obtained the three hexone bases, abundant tyrosine and very little leucine. On cleavage with barium hydoxide he obtained only lysine, besides tyrosine and glycocoll in larger amounts.

Fibroin and **sericin** are the two chief constituents of raw silk. By the action of boiling water the sericin (silk gelatin) dissolves and can be obtained by a method suggested by BONDI,[4] while the more difficultly soluble fibroin remains undissolved in the shape of the original fiber. The sericin, whose sufficiently concentrated hot solution gelatinizes on cooling, is precipitated by mineral acids, several metallic salts, and by acetic acid and potassium ferrocyanide. The spider silk investigated by FISCHER [5] yielded fibroin but not sericin.

Fibroin is soluble in concentrated acids and alkalies and reprecipitable (in a modified form) on neutralization. It gives the biuret test and MILLON'S and ADAMKIEWICZ'S reactions, the last only faintly. Fibroin has an especially great interest because of the hydrolyses performed by FISCHER and his co-workers, and especially by the finding of the previously mentioned polypeptides by these workers. Of the cleavage products which characterize fibroin we must mention the large amount of glycocoll, alanine and tyrosine, and the very small amounts of hexone bases, besides the nearly complete absence of monamino-dicarboxylic acids. The quantity of the hydrolytic cleavage products of the three silk substances, in so far as they have been investigated, are given in the following table, which also includes the results for elastin, gelatin, and koilin:

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[1] Zeitschr. f. Biol., **33**.

[2] Wheeler and Jamieson, Amer. Chem. Journ., **33**; Wheeler, *ibid.*, **38**.

[3] Henze, Zeitschr. f. physiol. Chem., **38** and **51**.

[4] *Ibid.*, **34**.

[5] *Ibid.*, **53**.

	Elastin.[1]	Gelatin.[1]	Koilin.[3]	Sericin.[1]	Fibroin.[1]	Spider Silk.[5]
Glycocoll	25.75	16.5	1.2	0.15	36.0	35.13
Alanine	6.6	0.8	5.8	5.0	21.0	23.4
Valine	1.0	1.0	....	....	....	....
Leucine	21.1	2.1	13.2	....	1.5	1.76
Serine	....	0.4	....	6.6	1.6	....
Aspartic acid	+?	0.56	2.3	....	+	....
Glutamic acid	0.8	0.88	5.2	....	....	11.70
Cystine	....	....	0.74[7]	....	....	....
Phenylalanine	3.9	0.4	2.3	....	1.5	....
Tyrosine	0.34	0.0	5.4	5.0	10.5	8.20
Proline	1.7	5.2	5.5	....	+	3.68
Oxyproline	....	3.0	....	....	....	....
Tryptophane	....	....	....	....	....	....
Histidine	....	0.4	0.03[4]	4.0	+	} 5.24[6]
Arginine	0.3	9.3[2]	3.60[4]	....	1.0	
Lysine	....	2.75	1.64[4]	....	+	

[1] Cited from Abderhalden's Lehrbuch. d. physiol Chem., 1909.
[2] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**.
[3] K. B. Hofmann and F. Pregl, *ibid.*, **52**.
[4] E. von Knaffl-Lenz, *ibid.*, **52**.
[5] E. Fischer, *ibid.*, **53**.
[6] Calculated as arginine.
[7] This figure is somewhat uncertain.

C. Cleavage Products of Simple Proteins.

On the hydrolysis of proteins by the aid of acids, alkalies or by enzymes, cleavage products are obtained which represent various intermediary steps between the native proteins on one side and the simple cleavage products, the amino-acids, on the other side. Among these products we have for a long time known two chief groups which still retain, to a high degree, their protein character, namely, the albuminates and the proteoses (and peptones).

1. Albuminates.

Alkali and Acid Albuminates. The native proteins are modified by the action of sufficiently strong acids or alkalies. By the action of alkalies all native albuminous bodies are converted, with the elimination of nitrogen, or by the action of stronger alkali, with the extraction of sulphur also, into a new modification, called alkali albuminate, whose specific rotation is increased at the same time. If caustic alkali in substance or in strong solution be allowed to act on a concentrated proteid solution, such as blood-serum or egg-albumin, the alkali albuminate may be obtained as a solid jelly which dissolves in water on heating, and which is called "LIEBERKÜHN'S solid alkali albuminate." By the action of dilute caustic alkali solutions on dilute proteid solutions we have

alkali albuminates formed slowly at the ordinary temperature, but more rapidly on heating. These solutions may vary with the nature of the proteid acted upon, and also with the intensity of the action of the alkali, but still they have certain reactions in common.

If proteid is dissolved in an excess of concentrated hydrochloric acid, or if we digest a proteid solution acidified with 1–2 p. m. hydrochloric acid in the thermostat, or digest the proteid for a short time with pepsin-hydrochloric acid, we obtain new modifications of proteid which indeed may show somewhat varying properties, but have certain reactions in common. These modifications, which may be obtained in a solid gelatinous condition on sufficient concentration, are called acid albuminates or acid albumins, and sometimes syntonin, though we prefer to apply the term syntonin to the acid albuminate, which is obtained by extracting muscles with hydrochloric acid of 1 p. m.

The alkali and acid albuminates have the following reactions in common: They are nearly insoluble in water and dilute common-salt solution (see page 103), but they dissolve readily in water on the addition of a very small quantity of acid or alkali. Such a solution as nearly neutral as possible does not coagulate on boiling but is precipitated at the normal temperature on neutralizing the solvent by an alkali or an acid. A solution of an alkali or acid albuminate in acid is easily precipitated on saturating with NaCl, but a solution in alkali is precipitated with difficulty or not at all, according to the amount of alkali it contains. Mineral acids in excess precipitate solutions of acid as well as alkali albuminates. The nearly neutral solutions of these bodies are also precipitated by many metallic salts.

Notwithstanding this agreement in the reactions, the acid and alkali albuminates are essentially different, for by dissolving an alkali albuminate in some acid no acid albuminate solution is obtained, nor is an alkali albuminate formed on dissolving an acid albuminate in water by the aid of a little alkali. In the first case we obtain a combination of the alkali albuminate and the acid soluble in water, and in the other case a soluble combination of the acid albuminate with the alkali added. The chemical process in the modification of proteids with an acid is essentially different from the modification with an alkali, hence the products are of a different kind. The alkali albuminates are relatively strong acids. They may be dissolved in water with the aid of $CaCO_3$, with the elimination of CO_2, which does not occur with typical acid albuminates, and they show in opposition to the acid albuminates also other variations which stand in connection with their strongly marked acid nature. Dilute solutions of alkalies act more energetically on proteids than do acids of corresponding concentration. In the first case a part of the nitrogen and often also the sulphur, is split off, and from this property we may

obtain an alkali albuminate by the action of an alkali upon an acid albuminate; but we cannot obtain an acid albuminate by the reverse reaction (K. MÖRNER [1]). This does not exclude the possibility that by the action of strong acids products can be obtained which perhaps correspond to those products obtained by the action of less stronger alkali.

The preparation of the albuminates has been given above. The corresponding albuminate obtained by the action of alkalies or acids upon a proteid solution may be precipitated by neutralizing with acid or alkali. The washed precipitate is dissolved in water by the aid of a little alkali or acid, and again precipitated by neutralizing the solvent. If this precipitate, which has been washed in water, is treated with alcohol and ether, the albuminate will be obtained in a pure form.

In the preparation of acid as well as of alkali albuminates, proteoses and the nearly related albuminates are formed. The *"alkali albumose"* obtained by MAAS [2] belongs to this class. The *lysalbinic acid* and *protalbinic acid* obtained by PAAL [3] from ovalbumin are likewise alkali albuminates. *Desaminoalbuminic acid* is an alkali albuminate which SCHMIEDEBERG [4] obtained by the action of such weak alkali that a part of the nitrogen was evolved but the quantity of sulphur remained the same. The proteid combination obtained by BLUM [5] by the action of formol on proteid and called by him *protogen*, has similarities with the alkali albuminates in regard to solubilities and precipitation, but is not identical therewith.

2. Proteoses and Peptones.

Peptones were formerly designated as the final products of the decomposition of protein bodies by means of proteolytic enzymes in so far as these final products are still true proteins, while the intermediate products produced in the peptonization of proteins, in so far as they are not substances similar to albuminates, were designated as proteoses (albumoses, or propeptones). Proteoses and peptones may also be produced by the hydrolytic decomposition of the proteins with acids or alkalies, and by the putrefaction of the same. They may also be formed in very small quantities as by-products in the investigations of animal fluids and tissues, and the question as to the extent to which these exist preformed under physiological conditions requires very careful investigation.

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[1] Pflüger's Arch., **17**.

[2] Zeitschr. f. physiol. Chem., **30**.

[3] Ber. d. d. chem. Gesellsch., **35**.

[4] Arch. f. exp. Path. u. Pharm., **39**.

[5] Blum, Zeitschr. f. physiol. Chem., **22**. The older investigations of Loew may be found in Maly's Jahresber., 1888. On the action of formaldehyde see also Benedicenti, Arch. f. (Anat. u.) Physiol., 1897; S. Schwarz, Zeitschr. f. physiol. Chem., **30**; Bliss and Novy, Journ. of Exper. Med., **4**.

Between the peptone, which represents the final cleavage product, and the proteose, which stands closest to the original protein, we have undoubtedly a series of intermediate products. Under such circumstances it is a difficult problem to try to draw a sharp line between the peptone and the proteose group, and it is just as difficult to define our conception of peptones and proteoses in an exact and satisfactory manner.

In the past we used to consider the peptones as the end products in the hydrolysis, they still being true proteins, but we must call attention to the fact that since that time we have learned of polypeptide-like cleavage products of the proteins, and also that polypeptides have been prepared synthetically. With this in mind it is not possible to say what we understand by the conception true proteid, and also that there possibly exists a large number of intermediary steps between the original modified proteid and the simplest cleavage products. There is no doubt that those bodies which have been called proteoses and peptones are chiefly mixtures; and the question has been proposed by ABDERHALDEN [1] whether it is not best to drop the conception of proteoses and to call all products precipitable by ammonium sulphate, etc., and previously described as proteoses, peptones.

Although there is much in favor of such a proposition, still on account of the great importance which the conception of the proteoses has generally received, it is probably too early to drop the question of proteoses entirely from a text-book, and we will therefore, as in the past editions, discuss the historical development of the proteoses and peptones in the ordinary sense.

The **proteoses** (or **albumoses**) used to be considered as those protein bodies whose neutral or faintly acid solutions do not coagulate on boiling and which, to distinguish them from peptones, were characterized chiefly by the following properties: The watery solutions are precipitated at the ordinary temperature by nitric acid, as well as by acetic acid and potassium ferrocyanide, and this precipitate has the peculiarity of disappearing on heating and reappearing on cooling. If a proteose solution is saturated with NaCl in substance, the proteose is partly precipitated in neutral solutions, but on the addition of acid saturated with salt it is more completely precipitated. This precipitate, which dissolves on warming, is a combination of the proteose with the acid.

We formerly designated as **peptones** those protein bodies which are readily soluble in water and which are not coagulated by heat, whose solutions are precipitated neither by nitric acid, nor by acetic acid and potassium ferrocyanide, nor by NaCl and acid.

The reactions and properties which the proteoses and peptones have

[1] Oppenheimer's Handb. der Biochem., Bd. **1**, 1908.

in common were formerly considered as the following: They all give the color reactions of the proteins, but with the biuret test they give a more beautiful red color than the ordinary proteins. They are precipitated by ammoniacal lead acetate, by mercuric chloride, tannic, phosphotungstic, and phosphomolybdic acids, by potassium-mercuric iodide and hydrochloric acid, and also by picric acid. They are precipitated but not coagulated by alcohol, that is, the precipitate obtained is soluble in water even after being in contact with alcohol for a long time. The proteoses and peptones also have a greater diffusive power than native proteins, and the diffusive power is greater the nearer the questionable substance stands to the final product, the now so-called true peptone.

These old views have gradually undergone an essential change. After HEYNSIUS'[1] observation that ammonium sulphate was a general precipitant for proteins, and for peptones in the old sense, KÜHNE and his pupils[2] proposed this salt as a means of separating proteoses and peptones. Those products of digestion which separate on saturating their solution with ammonium sulphate, or can indeed be salted out at all, are considered by KÜHNE and also by most of the modern investigators as proteoses, while those which remain in solution are called peptones or true peptones. These true peptones are formed in relatively large amounts in pancreatic digestion, while in pepsin digestion they are formed only in small quantities or after prolonged digestion.

According to SCHÜTZENBERGER and KÜHNE[3] the proteins yielded two chief groups of new protein bodies when decomposed by dilute mineral acids or with proteolytic enzymes; of these the *anti group* shows a greater resistance to further action of the acid and enzyme than the other namely, the *hemi group*. These two groups are, according to KÜHNE, united in the different proteoses, even though in various relative amounts, and each proteose contains the anti as well as the hemi group. The same is true for the peptone obtained in pepsin digestion, hence he calls it *amphopeptone*. In tryptic digestion a cleavage of the amphopeptone takes place into *antipeptone* and *hemipeptone*. Of these two peptones the hemipeptone is further split into amino-acids and other bodies while the antipeptone is not attacked. By the sufficiently energetic action of trypsin only one peptone is at last obtained—the so-called antipeptone.

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[1] Pflüger's Archiv, **34**.

[2] See Kühne, Verhandl. d. naturhistor. Vereins zu Heidelberg (N. F.), **3**; J. Wenz, Zeitschr. f. Biologie, **22**; Kühne and Chittenden, Zeitschr. f. Biologie, **22**; R. Neumeister, *ibid.*, **23**; Kühne, *ibid.*, **29**.

[3] Schützenberger, Bull. de la Soc. chimique de Paris, **23**; Kühne, Verhandl. d. naturhist. Vereins zu Heidelberg (N. F.), **1**, and Kühne and Chittenden, Zeitschr. f. Biologie, **19**. See also Paal, Ber. d. deutsch. chem. Gesellsch., **27**.

KÜHNE and his pupils, who have conducted extensive investigations on the proteoses and peptones, classify the various proteoses according to their different solubilities and precipitation properties. In the pepsin digestion of fibrin[1] they obtained the following proteoses: (*a*) *Heteroproteose*, insoluble in water but soluble in dilute salt solution; (*b*) *Protoproteose*, soluble in salt solution and water. These two proteoses are precipitated by NaCl in neutral solutions, but not completely. Heteroproteose may, by being in contact with water for a long time or by drying, be converted into a modification, called (*c*) *Dysproteose*, which is insoluble in dilute salt solutions. (*d*) *Deuteroproteose* is a proteose which is soluble in water and dilute salt solution and which is incompletely precipitated from acid solution by saturating with NaCl, and is not precipitated from neutral solutions. This precipitate is a combination of the proteose with acid (HERTH[2]). The deuteroproteose is essentially the same thing that BRÜCKE has designated as peptone.

The proteoses obtained from different protein bodies do not seem to be identical, but differ in their behavior to precipitants. Special names have been given to these various proteoses according to the mother-protein, namely, *albumoses*, *globuloses*, *vitelloses*, *caseoses*, *myosinoses*, *elastoses*, etc. These various proteoses are further distinguished, as *proto-*, *hetero-*, and *deuterocaseoses*, for example. CHITTENDEN[3] has suggested the common name proteoses for the products formed intermediary between the proteins and peptones in the digestion of animal and vegetable proteins. We have made use of it in this sense in preference to the word albumose (which is used in the German and by some other writers), but which will be used in this book as indicating the intermediary products in the hydrolysis of albumins and not as a general term. Certain proteoses have also been obtained in a crystalline state (SCHRÖTTER).

NEUMEISTER[4] designates as *atmidalbumose* that body which is obtained by the action of superheated steam on fibrin. At the same time he also obtained a substance called *atmidalbumin*, which stands between the albuminates and the proteoses.

Of the soluble proteoses NEUMEISTER designates the protoproteose and heteroproteose as *primary proteoses*, while the deuteroproteoses,

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[1] See Kühne and Chittenden, Zeitschr. f. Biologie, **20**.

[2] Monatshefte f. Chem., **5**.

[3] Kühne and Chittenden, Zeitschr. f. Biologie, **22** and **25**; Neumeister, *ibid.*, **23**; Chittenden and Hartwell, Journ. of Physiol., **11** and **12**; Chittenden and Painter, Studies from the Laboratory, etc., Yale University, **2**, New Haven, 1887; Chittenden, *ibid.*, **3**; Sebelien, Chem. Centralblatt, 1890; Chittenden and Goodwin, Journ. of Physiol., **12**.

[4] Zeitschr. f. Biologie, **26**. See also Chittenden and Meara, Journ. of Physiol., **15**, and Salkowski, Zeitschr. f. Biologie, **34** and **37**.

which are closely allied to the peptones, he calls *secondary proteoses*. As essential differences between the primary and secondary proteoses he suggests the following:[1] The primary proteoses are precipitated by nitric acid in salt-free solutions, while the secondary proteoses are precipitated only in salt solutions, and certain deuteroproteoses, such as deuterovitellose and deuteromyosinose, are precipitated by nitric acid only in solutions saturated with NaCl. The primary proteoses are precipitated from neutral solutions by copper-sulphate solution (2 : 100), and by NaCl in substance, while the secondary proteoses are not. The primary proteoses are completely precipitated from a solution saturated with NaCl by the addition of acetic acid saturated with salt, while the secondary proteoses are only partly precipitated. The primary proteoses are readily precipitated by acetic acid and potassium ferrocyanide, while the secondary are only incompletely precipitated after some time. The primary proteoses are also, according to PICK,[2] completely precipitated by ammonium sulphate (added to one-half saturation), while the secondary proteoses remain in solution.

The true peptones, as they were formerly considered to be, are exceedingly hygroscopic, and if perfectly dry, sizzle like phosphoric anhydride when treated with a little water. They are exceedingly soluble in water, diffuse more readily than the proteoses, and are not precipitated by ammonium sulphate. In contradistinction to the proteoses, the true peptones are not precipitated by nitric acid (even in solutions saturated with salt), by sodium chloride and acetic acid saturated with salt, potassium ferrocyanide and acetic acid, picric acid, trichloracetic acid, potassium-mercuric iodide, and hydrochloric acid. They are precipitated by phosphotungstic acid, phosphomolybdic acid, corrosive sublimate (in the absence of neutral salts), absolute alcohol, and tannic acid, but the precipitate may redissolve on the addition of an excess of the precipitant. As an important difference between amphopeptone and antipeptone we must also mention that the former gives MILLON'S reaction, while the antipeptone does not.

In regard to the precipitation by alcohol we must call attention to the observations of FRÄNKEL that not only are the acid combinations of peptone (PAAL) soluble in alcohol, but also the free peptone, and FRÄNKEL has even suggested a method of preparation based on this behavior. SCHRÖTTER [3] has also prepared crystalline proteoses which were soluble in hot alcohol, especially methyl alcohol.

The views on the hydrolytic cleavage products of peptic and tryptic digestion which were accepted until a few years ago have recently been considerably modified in several points.

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[1] Neumeister, Zeitschr. f. Biologie, **24** and **26**.

[2] Zeitschr. f. physiol. Chem., **24**.

[3] Fränkel, Zur Kenntnis der Zerfallsprodukte des Eiweisses bei peptischer und tryptischer Verdauung, Wien, 1896; Schrötter, Monatshefte f. Chem., **14** and **16**.

The older view that in peptic digestion only proteoses and peptones, but no simpler cleavage products, are formed, has been shown not to be true. The works of ZUNZ, PFAUNDLER, SALASKIN, LAWROW, LANGSTEIN,[1] and others have shown that by very lengthy digestion simpler products can be produced, some whose nature is still unknown, while others are known, such as alanine, leucine, leucinimide, valine, aspartic and glutamic acids, phenylalanine, tyrosine, proline and lysine, and on further cleavage indeed also oxyphenylethylamine, tetra- and pentamethylenediamine. It has not been possible to cause a disappearance of the biuret reaction, and the occurrence of tryptophane is somewhat disputed. MALFATTI obtained tryptophane in peptic digestion only when he used a certain apparently impure preparation of pepsin, and on using pepsin purified according to PEKELHARING it was absent. According to PEKELHARING,[2] purified pepsin also yields tryptophane when the solution is rich in pepsin, and also when the acidity is not too strong, in the presence of small amounts of pepsin.

In connection with the above-mentioned experimental results it must be remarked that not all the products found, for example the oxyphenylethylamine and the diamines, are produced by the action of pepsin, but rather by the action of other enzymes. In certain cases, undoubtedly, impure pepsin was used, or indeed autodigestion of the stomach was carried on, and the action of other enzymes was not excluded. In other cases the digestion with pepsin and considerable acid (even 1 per cent H_2SO_4) was continued for a very long time, indeed for an entire year, without controlling the influence of the acid alone upon the proteoses. The question as to the hydrolysis with the splitting off of amino-acids by the continuous action of acid alone is still the subject of dispute.

KÜHNE'S view that in tryptic digestion a peptone, so-called antipeptone, always remains which cannot be further split is not strictly true. By sufficiently long autodigestion of the pancreas, KUTSCHER[3] was able to obtain, as final products, a mixture of digestion products which failed to respond to the biuret test, and the same results have been obtained by others. In this connection we must remark that the pure antipeptone (see below), isolated by SIEGFRIED, could be split by trypsin only with great difficulty, and also that the complete disappearance of the biuret reaction in tryptic digestion does not show that a complete decomposition into amino-acids has taken place. According to E. FISCHER and ABDERHALDEN,[4] polypeptide-like bodies are produced, especially in

[1] Zunz, Zeitschr. f. physiol. Chem., **28**, and Hofmeister's Beiträge, **2**; Pfaundler, Zeitschr. f. physiol. Chem., **30**; Salaskin, *ibid.*, **32**; Salaskin and Kowalewsky, *ibid.*, **38**; Lawrow, *ibid.*, **33**; Langstein, Hofmeister's Beiträge, **1** and **2**.

[2] Malfatti, Zeitschr. f. physiol. Chem., **31**; Pekelharing, Archives d. scienc. biolog. de St. Pétersbourg, **11**; Pawlow Festband.

[3] Zeitschr. f. physiol. Chem., **25**, **26**, **28**, and Die Endprodukte der Trypsinverdauung, Habilitationsschrift Strassburg, 1899.

[4] Zeitschr. f. physiol. Chem., **39**.

tryptic digestion, and these bodies resist the prolonged action of the enzyme, but yield several different amino-acids on hydrolytic cleavage by acids. The same is probably also true for peptic digestion (see below), and the difference in the digestive products between pepsin and trypsin digestion consists, essentially, only in that, in the first case the cleavage is slower and does not proceed so far, hence the biuret reaction remains and generally no formation of tryptophane takes place.

By the use of the methods specially worked out by the HOFMEISTER school, of fractionally salting out with ammonium sulphate or zinc sulphate or also by SIEGFRIED'S iron-alum method, numerous attempts to separate the various proteoses and peptones have recently been made by UMBER, ALEXANDER, PFAUNDLER, PICK, ZUNZ, SIEGFRIED and his pupils.[1] Not only have we learned by these methods of a larger number of proteoses, but our older conception of the products formed primarily has been materially modified. Immediately at the commencement of digestion, even in peptic digestion, a splitting of the protein molecule into several complexes takes place. In opposition to the view of HUPPERT,[2] that the proteoses, in pepsin digestion, are always derived from the primarily formed acid albuminate, PICK and ZUNZ have shown that several proteoses, as well as acid albuminate, appear as primary products at the commencement of the digestion. According to GOLDSCHMIDT[3] a splitting off of proteoses and the formation of acid albuminate takes place simultaneously by the action of dilute acids alone. Besides the proteoses we also have, according to ZUNZ and PFAUNDLER, even at the beginning, other primary bodies, which cannot be salted out and which do not give the biuret reaction, but are in part precipitated by phosphotungstic acid. These little-known products seem to be intermediate between the peptones and the amino-acids, and they correspond probably to the polypeptide bodies obtained by FISCHER and ABDERHALDEN in tryptic digestion.

By fractional precipitation of WITTE'S peptone with ammonium sulphate PICK has obtained various chief fractions of proteoses. The first contains the proto- and heteroproteoses whose precipitation limit lies at 24–42 per cent saturation with ammonium sulphate solution, i.e., the presence of 24–42 cc. of the saturated ammonium sulphate solution in 100 cc. of the liquid. Then follows a fraction A at 54–62 per cent saturation, then a third fraction B, with 70–95 per cent saturation, and finally fraction C, which precipitates from the saturated solution on acidification with sulphuric acid saturated with the salt.

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[1] Umber, Zeitschr. f. physiol. Chem., **25**; Alexander, *ibid.*, **25**; Pfaundler, *ibid.*, **30**; Zunz, *ibid.*, **28**, and Hofmeister's Beiträge, **2**; Pick, *ibid.*, **2**, and Zeitschr. f. physiol. Chem., **24** and **28**; Siegfried, see footnote 2, page 135.

[2] Schütz and Huppert, Pflüger's Arch., **80**.

[3] F. Goldschmidt, Ueber die Einwirkung von Säuren auf Eiweissstoffe, Inaug.-Diss. Strassburg, 1898.

The hetero- and protoproteoses are not, according to our present views, the only primary proteoses. In the proteose fraction obtained on saturating with ammonium sulphate in neutral liquids, which should contain secondary proteoses only, primary proteoses such as the *glucoproteose* (PICK), which contains a carbohydrate group and the so-called *synproteose* (HOFMEISTER [1]) occur. It is no longer sufficient to consider an unequal ability to be salted-out, as an essential difference between the primary and secondary proteoses.

There is no doubt that there exists a large number of so-called proteoses having various precipitation properties, and different other properties and new differences appear in their investigation according to different methods. For example RONA and MICHAELIS [2] find that certain proteoses are precipitated by mastic emulsion while others are not. Those that are precipitable by mastic, can all be salted out, while all those that can be salted out are not all precipitated by mastic. The hetero- and protoproteoses act, according to ZUNZ [3] like strong protection colloids toward colloidal gold, which is not the case with the others, and also, according to this worker, the so-called proteoses are more readily precipitated by chondroitin-sulphuric acid and acetic acid than the so-called secondary proteoses. According to HUNTER [4] only the primary proteoses are precipitated by protamines while the secondary are not. It is also possible that numerous intermediary members exist between those proteoses which stand close to the original protein and those that are further removed. The difficulties in isolation and purification of these different members are so very great that the proteoses thus far isolated must not be considered as chemical individuals. Under these circumstances a more detailed discussion of the properties of the various proteoses thus far isolated is without interest.

It would be of great interest if certain differences in the chemical structure of the different proteoses could be determined with certainty. Such differences are claimed to have been found in certain cases. Thus HART has found that the heteroproteose (from muscle syntonin) was considerably richer in arginine and poorer in histidine than the protoproteose, and PICK has also found marked differences between the hetero- and proto-proteose from fibrin. The hetero-proteose yields very little tyrosine and indol but abundant leucine and glycocoll, and about 39 per cent of the total nitrogen in a basic form. The protoproteose,

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[1] Ueber Bau und Gruppirung der Eiweisskörper, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 783.

[2] Biochem. Zeitschr., **3**.

[3] Arch. internat. d. Physiol., **1** and **5**, and Bull. Soc. Scienc. med. et natur. Bruxelles, **64**.

[4] Journ. of Physiol., **37**.

according to PICK, on the contrary yields considerable tyrosine and indol, only little leucine but no glycocoll, and contains only about 25 per cent basic nitrogen. FRIEDMANN, HART, and LEVENE have obtained very similar results in regard to the quantity of basic nitrogen in the two proteoses, although LEVENE as well as ADLER [1] did not find the same results as PICK in regard to the amounts of monamino-acids in the two proteoses. These divergent results may be explained by the fact that they were not working with pure substances, but rather with mixtures. According to HASLAM [2] the so-called protoproteoses is a mixture of two proteoses which he designates α and β protoproteose which have different precipitation properties with alcohol and with one-half saturation with ammonium sulphate.

According to PICK the heteroproteose is also more resistant toward trypsin digestion than the protoproteose, a behavior which coincides with KÜHNE'S view of a resistant atomic complex, an antigroup, in the protein bodies. KÜHNE and CHITTENDEN [3] regularly obtained on the tryptic digestion of heteroproteose a separation of so-called antialbumid, a body which is attacked with great difficulty in tryptic digestion, but which separates as a jelly-like mass and which is richer in carbon (57.5–58.09 per cent), but poorer in nitrogen (12.61–13.94 per cent), than the original protein. The occurrence of such resistant complexes in digestion has also been repeatedly observed.

This antialbumid has recently attracted further attention, because as first found by DANILEWSKY and other investigators, OKUNEW, SAWJALOW, LAWROW, and SALASKIN and KURAJEFF, have further shown, that solutions of rennin, gastric juice, pancreatic juice, and papain cause a coagulum in not too dilute proteose solutions. These coagula, called *plasteines* (coagulum by rennin) by SAWJALOW, and *coaguloses* (coagulum by papain) by KURAJEFF,[4] are similar in many respects to antialbumid, having a higher content of carbon (57–60 per cent) and nitrogen (13–14.6 per cent). In other cases the quantity of carbon as well as nitrogen is lower (LAWROW).

We cannot for the present make any positive statement as to the importance and mode of formation of the coaguloses or plasteins. It

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[1] Hart, Zeitschr. f. physiol. Chem., **33**; Pick, *ibid.*, **28**; Friedmann, *ibid.*, **29**; Levene, Journ. of Biol. Chem., **1**; R. Adler, Die Heteroalbumose und Protalbumose des Fibrins. Dissert. Leipzig, 1907.

[2] Journ. of Physiol., **32** and **36**.

[3] Kühne and Chittenden, Zeitschr. f. Biologie, **19**, **20**.

[4] The works of Danilewsky and Okunew are cited and reviewed in the following: Sawjalow, Pflüger's Arch., **85**, and Centralbl. f. Physiol., **16**; and Zeitschr. f. physiol. Chem., **54**; Lawrow and Salaskin, Zeitschr. f. physiol. Chem., **36**; Lawrow, *ibid.*, **51**, **53** and **56**; Kurajeff, Hofmeister's Beiträge, **1** and **2**; see also Sacharow, Biochem. Centralbl., **1**, 233; Levene and v. Slyke, Biochem. Zeitschr., **13**.

is rather generally admitted that they are formed by a synthesis. According to SAWJALOW a plastein is not formed from a proteose alone, but always from a mixture of these. LAWROW claims that they may be produced from proteoses as well as from polypeptide substances, and correspondingly we must differentiate between the coaguloses or coagulosogens from the proteose group *coaproteoses*, and from the polypeptide group or *coapeptides*. The latter yield on hydrolysis chiefly monaminoacids while the first yield also basic nitrogenous products. Perhaps the plasteinogen investigated by BAYER,[1] which essentially differs from the true proteid in its elementary composition as well as from other coaguloses, belongs to the coapeptides.

The different behavior on saturating their solution with ammonium sulphate has been generally used, as above remarked, for years to differentiate between the proteoses and peptones. Those precipitable by this salt were called proteoses, and those not were called peptones. This method of division, which never had sufficient support and which was perfectly arbitrary, cannot be considered at the present time. We know now, thanks to the works of EMIL FISCHER and his co-workers, that there are polypeptides either prepared artificially or found among the cleavage products of the proteins, which are precipitated by ammonium sulphate. At the present it is generally conceded that the peptones in the ordinary sense are only a mixture of different bodies. The chief step in these investigations must be the isolation from this mixture of unit bodies with definite chemical characteristics. Of such bodies, besides the polypeptides previously mentioned and studied by FISCHER and others, we must mention the products isolated by SIEGFRIED and his pupils.[2]

These so-called peptones are in part peptic-peptones and partly tryptic-peptones, and some are prepared from proteid (fibrin) and others from gelatin. The tryptic fibrin-peptones are antipeptones in KÜHNE'S sense because they are very resistant to the further action of trypsin. They are according to NEUMANN simultaneously bibasic acids and monoacidic bases. They give the biuret reaction, but not MILLON'S reaction; they contain no tyrosine and yield on hydrolysis, arginine, lysine, glutamic acid, and it seems also aspartic acid. A peptic-glutin peptone isolated by SIEFGRIED and SCHEERMESSER yielded arginine, lysine, glutamic acid and glycocoll. SIEGFRIED has given proof in several ways as to the purity and unity of the peptones isolated by him.

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[1] Hofmeister's Beiträge, **4**; see also L. Rosenfeld, *ibid.*, **9**; J. Lukomnik, *ibid.*, **9** and F. Micheli, Biochem. Centralbl., **6**, p. 562.

[2] The works of Siegfried and his pupils, Fr. Müller, Borkel, Mühler, Krüger, Scheermesser and Neumann may be found in Arch. f. (Anat. u.) Physiol., 1894 and Zeitschr. f. physiol. Chem., **21**, **41**, **43**, **45**, **48** and **50**.

In another manner, namely by fractional precipitation with metallic salts, especially with mercuric-potassium iodide and the preparation of phenylisocyanate compounds, HOFMEISTER and his pupils STOOKEY, RAPER and ROGOZINSKI [1] have isolated peptones or polypeptide-like bodies from blood proteid. One of these, called arginine-histidine peptone, yielded arginine and histidine as basic hydrolytic products while another yielded chiefly lysine as basic product and hence was called lysine-peptone.

From glutin-peptone, SIEGFRIED, on warming with hydrochloric acid, obtained a base, $C_{21}H_{39}N_9O_8$, which can also be directly obtained from gelatin. This he calls a *kyrin*, because it is to be considered as a basic protein nucleus, and he calls this special one *glutokyrin*. The glutokyrin gives the biuret reaction and is considered as a basic peptone. On complete hydrolytic cleavage it yields arginine, lysine, glutamic acid, and glycocoll. Of the total nitrogen two-thirds belongs to the bases and one-third to the amino-acids.

Recently he with O. PILZ on further hydrolysis has prepared a β-glutokyrin, which only yielded arginine, lysine and glutamic acid. Similar basic nuclei, *protokyrins*, have recently been obtained by SIEGFRIED [2] from fibrin and casein, using the same method. Caseinokyrin gives a non-crystalline sulphate, but a crystalline phosphotungstate. The free caseinokyrin has an alkaline reaction, gives the biuret test, and its composition corresponds to the formula $C_{23}H_{47}N_9O_8$. It yields arginine, lysine, and glutamic acid on cleavage. The basic nitrogen amounts to about 85 per cent of the total nitrogen, and caseinokyrin, whose unit nature is defended by SIEGFRIED [3] against the opinions of SKRAUP, ZWERGER and WITT,[4] behaves in this respect like a protamine.

Among the known cleavage products of proteins, arginine is the only one which, up to the present, is never absent, and for this reason we designate as proteins only those atomic complexes which contain, besides chained monamino-acids, also arginine, or, more simply, show the previously mentioned imide bindings. Hence caseinokyrin, which yields only arginine, lysine and glutamic acid, and scombrin (see below), which yields only arginine, proline, and alanine, are the simplest known proteins.

Scombrin belongs to the previously mentioned group of protamines which, according to KOSSEL,[5] are formed by a successive cleavage of the typical protein. The occurrence of basic protokyrins in the hydrolytic

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[1] Hofmeister's Beiträge, **7**, **9**, and **11**.

[2] Kgl. Sächs. Ges. d. Wiss., Math.-Phys. Klasse, 1903, and Zeitschr. f. physiol. Chem., **43**, with Pilz., *ibid.*, 58.

[3] Zeitschr. f. physiol. Chem., **48** and **50**.

[4] Monatash. f. Chem., **26** and **27**.

[5] Zeitschr. f. physiol. Chem., **44**.

cleavage of genuine proteins like gelatin has given valuable support to KOSSEL'S theory as to a basic nucleus in the protein bodies.

On account of the cleavage taking place in digestion, the digestive products should have a lower molecular weight than the original protein. This is really the case. As these determinations have been made upon impure substances or mixtures, the results [1] obtained are only of little value. The same is true for the elementary analysis of the proteoses and peptones.[2]

Besides the behavior in the salting-out process, attempts have been made to find other points of difference between the peptones and proteoses. SCHRÖTTER and FRÄNKEL [3] consider the sulphur content as a pronounced point of difference. The peptones, according to them, are free from sulphur, while the proteoses, on the contrary, contain sulphur. FRÄNKEL has been able to find only one proteose (in KÜHNE'S sense) which did not contain sulphur.

In the preparation and separation of various proteoses and peptones all precipitable protein is always removed first by neutralization and then by boiling. The proteoses may then be separated from the peptones by means of ammonium sulphate according to KUHNE'S method, and divided into different fractions according to the method of PICK and the HOFMEISTER school. The separation and preparation of pure hetero- and protoproteoses can be best performed by the method suggested by PICK, but this method, as well as that with ammonium sulphate, gives good results only when the precautions suggested by HASLAM [4] are carefully followed. We can here only refer to the cited works of Kühne and co-workers, of E. ZUNZ and especially those of the HOFMEISTER and the SIEGFRIED schools. In regard to the literature on the detection of proteoses and peptones in animal fluids we refer to Chapters VI and XV.

If we wish to detect the presence of so-called true peptone, by means of the biuret reaction in a solution saturated with ammonium sulphate, we add a slight excess of a concentrated solution of caustic soda and cool, and then add a two per cent solution of copper sulphate drop by drop, after the sodium sulphate has separated out.

In the quantitative estimation of proteoses and peptones we make use of the nitrogen estimation, the biuret test (colorimetric), and the polarization method. These methods do not give exact results.

The polypeptides have had their most important properties discussed on pages 85-89, and of the cleavage products of the proteins only the amino-acids remain to be discussed.

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[1] Sabanejew, Ber. d. d. chem. Gesellsch., **26**, 385; Paal, *ibid.*, **27**, 1827; Sjöqvist, Skand. Arch. f. Physiol., **5**.

[2] Elementary analyses of proteoses and peptones will be found in the works of Kühne and Chittenden and their pupils, cited in footnote 3, p. 129; also by Herth, Zeitschr. f. physiol. Chem., **1**, and Monatshefte f. Chem., **5**; Maly, Pflüger's Arch. **9**, **20**; Henninger, Compt. rend., **86**; Schrötter, l. c., Paal, l. c.

[3] Schrötter, Monatshefte f. Chem., **14** and **16**; Fränkel, Zur Kenntnis der Zerfallsprodukte des Eiweiss bei peptischer und tryptischer Verdauung, Wien, 1896.

[4] Journ. of Physiol., **32** and **36**.

3. The Amino Acids.[1]

Glycocoll (amino-acetic acid), $C_2H_5NO_2 = \begin{matrix} CH_2(NH_2) \\ COOH \end{matrix}$, also called glycine or gelatin sugar, is found in the muscles of the invertebrates, but has chief interest as a hydrolytic decomposition product of protein bodies, especially fibroin, spider-silk elastin, gelatin, and spongin, as well as of hippuric acid and glycocholic acid. It is also formed in the decomposition of uric acid, xanthine, guanine, and adenine.

Glycocoll forms colorless, often large, hard rhombic crystals or four-sided prisms. The crystals have a sweet taste and dissolve readily in cold water (4.3 parts). Glycocoll is insoluble in alcohol and ether and dissolves with difficulty in warm alcohol. It combines with acids and alkalies. With the latter compounds we must mention those with copper and silver. Glycocoll dissolves cupric hydroxide in alkaline liquids, but does not reduce at boiling heat. A boiling-hot solution of glycocoll dissolves freshly precipitated cupric hydroxide, forming a blue solution, which in proper concentration deposits blue needles of copper-glycocoll on cooling. The compound with hydrochloric acid is readily soluble in water but less soluble in alcohol.

Sörensen[2] finds that phosphotungstic acid does not precipitate glycocoll from dilute solutions but only from concentrated ones. By the action of gaseous HCl upon glycocoll in absolute alcohol, beautiful crystals are obtained of the hydrochloride of glycocoll ethyl ester, which melts at 144° C. and from which the glycocoll ethyl ester can be obtained by the method suggested by E. Fischer[3] for the separation of glycocoll from the other amino-acids. On shaking with benzoyl chloride and caustic soda, hippuric acid is formed, and this is also made use of in different ways in detecting and isolating glycocoll (Ch. Fischer, Gonnermann, Spiro[4]). The β-naphthalene-sulpho-glycine with a melting-point of 159°, the 4 nitro-tolulene-2-sulpho-glycine, melting at 180°, the phenylisocyanate compound, melting at 195°, and the α-naphthyl-isocyanate compound melting at 190.5–191.5° are also of importance.

Glycocoll can be best prepared from hippuric acid by boiling it with 4 parts dilute sulphuric acid (1:6) for ten to twelve hours. After cooling the benzoic acid is removed, the filtrate concentrated, the remaining benzoic acid removed by extracting with ether, the sulphuric acid pre-

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[1] In regard to the division of the animo-acids among the three chief groups of organic compounds we refer to page 85.

[2] Meddelelser, fraa Carlsberg-laboratoriet, **6**, 1905.

[3] Ber. d. d. chem. Geseilsch., **34**.

[4] Ch. Fischer, Zeitschr. f. physiol. Chem., **19**; Spiro, *ibid.*, **28**; Gonnermann, Pflüger's Arch., **59**.

cipitated by $BaCO_3$, and the filtrate evaporated to the point of crystallization. (In regard to its preparation from protein substances see below.)

Alanine (α-aminopropionic acid, $C_3H_7NO_2 = \begin{matrix} CH_3 \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$). The *d*-alanine is obtained in relatively small amounts from the true proteids, but in larger quantities from the albuminoids, especially from fibroin, spider-silk and elastin.

d-alanine has been prepared from *l*-serine by E. Fischer and K. Raske [1], and Fischer has also obtained it from racemic alanine by splitting the benzoyl combination or from *l*-alanine by splitting with yeast by Walden's reversion (see page 87).

Alanine generally crystallizes in needles or oblique rhombic columns It is very readily soluble in water, having a sweetish taste, and dissolves cupric hydroxide on boiling, producing a deep blue solution of a crystallizable copper salt. Alanine is insoluble in absolute alcohol. The rotation of alanine at 20° C. in aqueous solution is $(a)_D = +2.7°$ and for a solution in hydrochloric acid (9–10 per cent solution) is $(a)_D = +10.3°$.

The β-naphthalene-sulpho-*d*-alanine melts at 79–81°, the phenylisocyanate at 168°, and the naphthylisocyanate-alanine melts at 198° C.

Valine (α-amino-valeric acid) $C_5H_{11}NO_2 = \begin{matrix} CH_3\,CH_3 \\ \diagdown\ \diagup \\ CH \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$, has been detected several times among the cleavage products of protein substances, although only in small quantities. Kossel and Dakin obtained 4.3 per cent valine from salmine, and E. Fischer and Dörpinghaus [2] 5.7 per cent from horn substances. The acid isolated by H. and E. Salkowski [3] from putrefying proteid or gelatin seems to have been α-amino-*n*-valeric acid.

d-valine can be obtained as microscopic crystalline leaves. It is rather readily soluble in water and the solution has a faint sweetish taste and at the same time somewhat bitter. The solution has a rotation of $(a)_D = +6.42°$. The hydrochloric acid solution (20 per cent) shows, according to Fischer, a rotation of $(a)_D = +28.8°$. The copper salt, which forms leaves which are rather soluble in water, is very easily soluble in methyl alcohol (Schulz and Winterstein [4]).

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[1] Ber. d. d. chem. Gesellsch., **40**.

[2] Kossel and Dakin, Zeitschr. f. physiol. Chem., **41**; Fischer and Dörpinghaus, *ibid.*, **36**.

[3] Ber. d. d. chem. Gesellsch., **16** and **31**.

[4] Zeitschr. f. physiol. Chem., **35**.

The phenylisocyanate melts at 147°, and on boiling with 20-per cent hydrochloric acid for a short time, it is changed into *d*-phenylisopropyl hydantoin, which melts at 131–133° C.

Leucine (aminocaproic acid, or, more correctly, α-aminoisobutylacetic acid), $C_6H_{13}NO_2 = \begin{matrix} CH_3CH_3 \\ \diagdown\diagup \\ CH \\ | \\ CH_2 \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$, is produced from protein substances in their hydrolytic cleavage by proteolytic enzymes, by boiling with dilute acids or by fusing with alkali hydroxides, and by putrefaction. There are also observations that indicate that in the hydrolysis besides the ordinary leucine perhaps also normal leucine may be formed (HECKEL and SAMEC [1]).

Because of the ease with which leucine (and tyrosine) are formed in the decomposition of protein substances, it is difficult to decide positively whether these bodies when found in the tissues are constituents of the living body or are to be considered only as decomposition products formed after death. Leucine, it seems, has been found as a normal constituent of the pancreas and its secretion, in the spleen, thymus, and lymph glands, in the thyroid gland, in the salivary glands, in the kidneys and in the liver. It also occurs in the wool of sheep, in dirt from the skin (inactive epidermis), and between the toes, and its decomposition products have the disagreeable odor of the perspiration of the feet. It is found pathologically in atheromatous cysts, ichthyosis scales, pus, blood, liver, and urine (in diseases of the liver and in phosphorus poisoning). Leucine occurs often in invertebrates and also in the plant kingdom. On hydrolytic cleavage various protein substances yield different amounts of leucine, as shown in the tables given on pages 106, 107, 114 and 124. From the figures there given we call attention to the following: ERLENMEYER and SCHÖFFER obtained 36–45 per cent leucine from the cervical ligament, COHN obtained 32 per cent from casein, E. FISCHER and ABDERHALDEN 20 per cent from hæmoglobin, and FISCHER and DÖRPINHAUS 18.3 per cent from horn substance.[2]

The leucine obtained by cleavage of protein substances is generally *l*-leucine, which is levorotatory in water solution and dextrorotatory in acid solution. The leucine prepared synthetically by HÜFNER [3] from

[1] Heckel, Monatsh. f. Chem., **29**; Samec, *ibid.*, **29**.

[2] Erlenmeyer and Schöffer, cited from Maly, Chem. d. Verdauungssäfte, in Hermann's Handb. d. Physiol., **5**, Theil 2, p. 209; Cohn, Zeitschr. f. physiol. Chem., **22**; Fischer and his collaborators, *ibid.*, **36**.

[3] Journ. f. prakt. Chem. (N. F.), **1**.

isovaleraldehyde, ammonia, and hydrocyanic acid is optically inactive. Inactive leucine may also be prepared, as shown by E. SCHULZE and BOSSHARD,[1] by the cleavage of proteins with baryta at 160–180° C., because of a racemation, as the ordinary leucine is racemized on heating with baryta at this temperature. The *d-l*-leucine may be split into the two components by various means, especially by the preparation of the formyl combination.[2]

On oxidation the leucines yield the corresponding oxyacids (leucinic acids). Leucine is decomposed on heating, evolving carbon dioxide, ammonia, and amylamine. On heating with alkalies, as also in putrefaction, it yields valeric acid and ammonia.

Leucine crystallizes when pure in shining, white, very thin plates, usually forming round knobs or balls, either appearing like hyaline, or with alternating light and dark concentric layers which consist of radial groups of crystals. By slow heating, leucine melts and sublimes into white, woolly flakes, which are similar to sublimed zinc oxide. At the same time an odor of amylamine is developed. Quickly heated in a closed capillary tube, it melts with decomposition at 293–295°.

Leucine, as obtained from animal fluids and tissues is always impure, and is very easily soluble in water and rather easily in alcohol. Pure leucine is soluble with difficulty. Pure *l*- and *d*-leucine dissolve in 40–46 parts water, more readily in hot alcohol, but with difficulty in cold alcohol. The *d-l*-leucine is much less soluble. According to HABERMANN and EHRENFELD[3] 100 parts of boiling glacial acetic acid dissolve 29.23 parts of leucine. The specific rotation of *l*-leucine, dissolved in hydrochloric acid (20 per cent solution) is $(\alpha)_D = +15.6°$ according to FISCHER and WARBURG. In aqueous solution it is $(\alpha)_D = -10.42°$, according to EHRLICH and WENDEL.[4]

The solution of leucine in water is not, as a rule, precipitated by metallic salts. The boiling-hot solution may, however, be precipitated by a boiling-hot solution of copper acetate, and this fact is made use of in separating leucine from other substances. If the solution of leucine is boiled with sugar of lead and then ammonia be added to the cooled solution, shining crystalline leaves of leucine-lead oxide separate. Leucine dissolves cupric hydroxide, but does not reduce on boiling.

Leucine is readily soluble in alkalies and acids. It gives crystalline compounds with mineral acids. If leucine hydrochloride is boiled with alcohol containing 3–4 per cent HCl, long narrow crystalline prisms of

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[1] See Zeitschr. f. physiol. Chem., **9** and **10**.

[2] Fischer and Warburg, Ber. d. d. chem. Gesellsch., **38**.

[3] Zeitschr. f. physiol. Chem., **37**.

[4] Fischer and Warburg, Ber. d. d. chem. Gesellsch., **38**; Ehrlich and Wendel, Biochem. Zeitschr., **8**.

leucine-ethyl-ester hydrochloride, melting at 134° C., are formed (Röhmann). The same is produced by the action of gaseous HCl upon leucine in alcohol, and the free ethyl ester can be obtained from this by the method suggested by E. Fischer.[1]

The picrate of the leucine ester melts at 128°. The phenylisocyanate of *d-l*-leucine melts at 165° and its anhydride at 125° C. The α–naphthyl-isocyanate leucine melts at 163.5°, the naphthalene-sulpho-*l*-leucine at 68° C.

Leucine is recognized by the appearance of balls or knobs under the microscope, by its action when heated (sublimation test), and by its compounds, especially the hydrochloride and picrate of the ethyl ester and the phenylisocyanate compound of the racemic leucine obtained on heating with baryta water, the α-naphthylisocyanate compound and the β-naphthalene-sulpho-leucine. According to the method suggested by Lippich[2] the leucine can be transformed into isobutylhydantoin, having a melting point of 205°, by boiling with an excess of urea and baryta water. In the detection of leucine, it must be first isolated, and the preparation of the ethyl ester and distillation of the same are important in this regard. In order to avoid the difficulties which occur in the preparation of pure leucine, Ehrlich and Wendel[3] have suggested a new method.

Leucinimide, $C_{12}H_{22}N_2O_2 = \begin{matrix} C_4H_9.CH.NH.CO \\ CO.NH.CH.C_4H_9 \end{matrix}$, was first obtained by Ritthausen in the hydrolytic cleavage products on boiling proteins with acids, and subsequently by R. Cohn. Salaskin[4] obtained it in the peptic and tryptic digestion of hæmoglobin. As an anhydride of leucine (2.5-diacipiperazine) it is probably formed by a secondary change, from leucine.

It crystallizes in long needles and sublimes readily. The melting-point has not been found constant in the different cases. The leucinimide (3.6-diisobutyl-2.5-diacipiperazine) prepared synthetically by E. Fischer[5] from leucine-ethyl ester melted at 271° C.

Isoleucine (methyl-ethyl-α-amino-propionic acid) $C_6H_{13}NO_2 = \begin{matrix} CH_3C_2H_5 \\ \diagdown\diagup \\ CH \\ CHNH_2 \\ COOH \end{matrix}$

is an isomer of leucine discovered by F. Ehrlich, who first isolated it from the mother-liquor after removing the sugar from beet-sugar molasses. He also found it in the hydrolysis of several proteins, and

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[1] Röhmann, Ber. d. d. chem. Gesellsch., **30**; E. Fischer, *ibid.*, **34**.

[2] Ber. d. d. chem. Gesellsch., **39**.

[3] Biochem. Zeitschr., 8.

[4] Ritthausen, Die Eiweisskörper der Getreidearten, etc., Bonn, 1872; R. Cohn, Zeitschr. f. physiol. Chem., **22** and **29**; Salaskin, *ibid.*, **32**.

[5] Ber. d. d. chem. Gesellsch., **34**.

recently it has been found by others [1] among the products of hydrolysis of the proteins. It seems to be associated regularly with ordinary leucine, forming mixed crystals, which give an impression of a chemical combination and which are difficult to separate. On this account the earlier claims as to the quantity of leucine are somewhat uncertain, as they always refer to leucine containing isoleucine.

The constitution of isoleucine has been explained by EHRLICH through its relation to *d*-amyl alcohol. In the fermentation of sugar by yeast it yields *d*-amyl alcohol, and on the other hand, it can also be obtained, in a manner analogous to the synthesis of leucine, from *d*-amyl alcohol (as a mixture of isoleucine and alloisoleucine, the latter is levogyrate and has a different stereometric configuration from the isoleucine). The synthesis of isoleucine has been accomplished in other ways by EHRLICH, by BRASCH and FRIEDMANN and by BOUVEAULT and LOCQUIN.[2]

Isoleucine crystallizes in leaves or rods and plates of the rhombic form. It is more soluble in water than leucine (1:25.8). Its solutions have a bitter taste and are astringent. It is dextro-rotatory in aqueous as well as in acid solution. In aqueous solution it has a specific rotation of $(a)_D = +9.74°$ and in 20-per cent hydrochloric acid $(a)_D = +36.8°$. Like valine its copper salt is readily soluble in methyl alcohol. The benzoyl combination melts at 116–117°, the benzene sulphoisoleucine at 149–150°, the phenylisocyanate combination at 119–120°, and the naphthylisocyanate combination at 178° C.

Serine (α-amino-β-oxypropionic acid) $C_3H_7NO_3 = \begin{matrix} CH_2(OH) \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{matrix}$, was obtained by FISCHER and his collaborators as a cleavage product of several proteins, generally only in small quantities. The largest quantity, 6.6 per cent, was obtained by FISCHER and SKITA [3] from sericine; KOSSEL and DAKIN [4] obtained a still larger amount from salmine, namely 7.8 per cent. The racemic serine is the one generally obtained. From fibroin FISCHER [5] obtained a mixture of active and inactive serine anhydride from which he finally prepared *l*-serine by hydrolysis.

Synthetically *d-l*-serine has been prepared by FISCHER and LEUCHS

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[1] Felix Ehrlich, Ber. d. d. chem. Gesellsch., **37**; Winterstein and Pantanelli, Zeitschr. f. physiol. Chem., **45**.

[2] Ehrlich, Ber. d. d. chem. Gesellsch., **40** and **41**; Brasch and Friedmann, Hofmeister's Beiträge, **11**; Bouveault and Locquin, Compt. rend., **141**, and Bull. soc. chim. (3), **35**; Locquin, Bull. soc. chim. (4), **1**.

[3] Zeitschr. f. physiol. Chem., **35**.

[4] Fischer and Skita, Zeitschr. f. physiol. Chem., **35**; Kossel and Dakin, *ibid.*, **41**.

[5] Ber. d. d. chem. Gesellsch., **40**.

from ammonia, hydrocyanic acid and glycol aldehyde, and recently in other ways by ERLENMEYER, JR. and STOOP and by LEUCHS and GEIGER.[1] FISCHER and JACOBS[2] have prepared *l*-serine from *d-l*-serine by the preparation of the alkaloid salt of the *p*-nitro-benzoyl combination. On reduction serine is transformed into alanine, and on oxidation with nitrous acid it yields glyceric acid. The relation of serine to alanine, lactic acid and glyceric acid is evident from the following formulæ:

$CH_2(OH)$	CH_3	CH_3	$CH_2(OH)$
$\dot{C}H(NH_2)$	$\dot{C}H(NH_2)$	$\dot{C}H(OH)$	$\dot{C}H(OH)$
$\dot{C}OOH$	$\dot{C}OOH$	$\dot{C}OOH$	$\dot{C}OOH$
Serine	Alanine	Lactic acid	Glyceric acid

The *l*-serine crystallizes in thin leaves or crusts. It is rather readily soluble in water; the *d-l*-serine is soluble in 23 parts water at 20° C. The solution of *l*-serine has a sweet taste with an insipid after taste. The specific rotation in aqueous solution at 20° C. is $(\alpha)_D = -6.83°$ and the hydrochloric acid solution at 25° C. is $(\alpha)_D = +14.45°$. The *β*-naphthalene-sulpho-serine melts at 220° C. when anhydrous. The *l*-serine anhydride, which is identical with that obtained from fibroin, forms thin, colorless needles which melt at 247° with decomposition. Its specific rotation in aqueous solution at 25° C. $(\alpha)_D = -67.46°$.

Isoserine (*β*-amino-*α*-oxypropionic acid) has been prepared by ELLINGER from diamino-propionic hydrobromide and silver nitrite and by NEUBERG and SILBERMANN from the hydrochloric acid combination of diamino-propionic acid. Other syntheses have been made by NEUBERG and MAYER and by NEUBERG and ASCHER.[3]

Aspartic acid (aminosuccinic acid), $C_4H_7NO_4 = \begin{matrix} COOH \\ \dot{C}H(NH_2) \\ \dot{C}H_2 \\ \dot{C}OOH \end{matrix}$, has been obtained on the cleavage of protein substances by proteolytic enzymes as well as by boiling them with dilute mineral acids in quantities given in the tables on pages 106, 107, 114 and 124. This acid also occurs in secretions of sea-snails (HENZE[4]) and is very widely diffused in the vegetable kingdom as the amide ASPARAGINE (aminosuccinic-acid amide), which seems to be of the greatest importance in the development and formation of the proteins in plants. *d-l*-Aspartic acid has been prepared synthetically from fumaric acid and alcoholic ammonia.

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[1] Fischer and Leuchs, Ber. d. d. chem. Gesellsch, **35**; Erlenmeyer and Stoop, *ibid.*, **35**; Leuchs and Geiger, *ibid.*, **39**.

[2] Ber. d. d. chem. Gesellsch., **39**.

[3] Ellinger, Ber. d. d. chem. Gesellsch., **37**; Neuberg and Silbermann, *ibid.*, **37**; Neuberg and Mayer, Biochem., Zeitschr **3**; Neuberg and Ascher, *ibid.*, **6**.

[4] Ber. d. d. chem. Gesellsch., **34**.

l-Aspartic acid dissolves in 256 parts water at 16° C. and in 18.6 parts boiling water, and on cooling crystallizes as rhombic prisms, and its 4 per cent solution acidified with HCl has the rotation $(\alpha)_D = +25.7°$; in alkaline solution the acid is levo-rotatory. It forms with copper oxide a crystalline compound which is soluble in boiling-hot water and nearly insoluble in cold water, and which may be used in the preparation of the pure acid from a mixture with other bodies.

The benzoyl-*l*-aspartic acid melts at 184–185°. For identification we make use of the analysis of the free acid and the copper salt, as well as of the specific rotation.

Glutamic acid (α-aminoglutaric acid), $C_5H_9NO_4 = \begin{matrix} \dot{C}OOH \\ \dot{C}H(NH_2), \\ \dot{C}H_2 \\ \dot{C}H_2 \\ \dot{C}OOH \end{matrix}$, is obtained from the protein substances under the same conditions as the other mono-amino-acids (see tables on pages 106, 107, 114 and 124) and from the peptones (SIEGFRIED). HLASIWETZ and HABERMANN obtained 29 per cent from casein by cleavage with hydrochloric acid, while KUTSCHER could obtain only 1.8 per cent glutamic acid by cleavage with sulphuric acid. ABDERHALDEN and FUNK[1] did not find any such differences in the hydrolytic action of the two acids, and obtained only 10–11 per cent glutamic acid from casein. SKRAUP and TÜRK[2] obtained on the hydrolysis of casein with 33 per cent sulphuric acid at boiling temperature for eighteen hours about the same quantity of glutamic acid as on boiling for six hours with fuming hydrochloric acid, namely, 20.3 and 22.3 per cent glutamic acid hydrochloride. The considerable quantity of glutamic acid obtainable from certain vegetable proteins, as shown on page 107, is very remarkable. LEVENE and MANDEL[3] have found a strikingly large quantity of glutamic acid, namely 25 per cent, from a nucleoprotein from the spleen.

d-Glutamic acid crystallizes in rhombic tetrahedra or octahedra or in small leaves. It dissolves in 100 parts water at 16° C., and the solution tastes acid with a peculiar after-taste. It is insoluble in alcohol and in ether.

In water it has a rotation of $(\alpha)_D = +12.04°$ according to ANDRLIK.[4] Strong acids increase the rotation, and a 5 per cent solution of glutamic acid containing 9 per cent HCl has a rotation $(\alpha)_D = +31.7°$, while that obtained by heating with barium hydroxide is optically inactive. The

[1] Hlasiwetz and Habermann, Annal. d. chem. u. Pharm., **159**; Kutscher, Zeitschr. f. physiol. Chem., **28**; Abderhalden and Funk, *ibid.*, **53**.

[2] Monatsh. f. chem., **30**.

[3] Biochem. Zeitschr., **5**.

[4] See Biochem. Centralbl., **3**, p. 469.

d-glutamic acid forms a beautifully crystalline combination with hydrochloric acid, which is nearly insoluble in concentrated hydrochloric acid. This compound is used in the isolation of glutamic acid. On boiling with cupric hydroxide a beautiful crystalline copper salt, which is soluble with difficulty, is obtained. The benzoyl-*d*-glutamic acid melts at 130–132° C. The hydrochloride, the α-naphthylisocyanate of glutamic acid, which melts at 236–237° C., the analysis of the free acid, and the specific rotation are used in its detection.

As previously stated monamino-oxydicarboxylic acids have also been found among the cleavage products of the proteins. To these belong the following:

That **oxyaminosuccinic acid,** $C_{11}H_7NO_5$ occurs among the hydrolytic cleavage products of proteids has been shown to be probable by SKRAUP. This acid has been prepared synthetically by NEUBERG and SILBERMANN from diaminosuccinic acid and barium nitrite in sulphuric acid solution. **Oxyaminosuberic acid,** $C_8H_{15}NO_5$, has been detected by WOHLGEMUTH [1] in the cleavage products of a liver nucleoprotein.

Cystine, $C_6H_{12}N_2S_2O_4$ the disulphide of **cysteine** (α-amino-β-thiolactic acid), $\begin{matrix}CH_2—S—S—CH_2\\ \dot{C}H(NH_2) \quad \dot{C}H(NH_2)\\ \dot{C}OOH \quad \dot{C}OOH\end{matrix}$, was first obtained with certainty as a cleavage product of protein substances by K. MÖRNER, and then also by EMBDEN. KÜLZ [2] obtained it once as a product of tryptic digestion of fibrin. The quantities found by MÖRNER and BUCHTALA in the various proteins are given in the tables on pages 106, 107, 113 and 114.

According to NEUBERG and MAYER [3] two kinds of cystine occur in nature, namely, *stone-cystine*, designated β-cystine, and *protein-cystine*. Stone-cystine is the disulphide of β-amino-α-thiolactic acid, $\begin{matrix}\dot{C}H_2NH_2 \quad \dot{C}H_2NH_2\\ \dot{C}H—S—S—\dot{C}H\\ \dot{C}OOH \quad \dot{C}OOH\end{matrix}$.

The protein-cystine has been chiefly obtained from the protein substance, but also from calculi, while the stone-cystine has been obtained from urinary calculi only.

Many objections have been raised from many sides as to the correctness of this assumption. ROTHERA could not find any difference between the stone-cystine and the cystine prepared from hair, and FISCHER and SUZUKI, and recently also ABDERHALDEN, [4] arrived at similar results, which seems to place the existence of stone-cystine in doubt. The occurrence of two structurally isomeric cystines is not improbable, from certain observations of MÖRNER, but FRIEDMANN and BAER [5] have shown that these observations do not lead to this assumption,

[1] Skraup, Zeitschr. f. physiol. Chem., **42**; Neuberg and Silbermann, *ibid.*, **44**; Wohlgemuth, *ibid.*, **44**.

[2] K. Mörner, *ibid.*, **28**, **34**, and **42**; Embden, *ibid.*, **32**; Külz, Zeitschr. f. Biologie, **27**.

[3] Zeitschr. f. physiol. Chem., **44**.

[4] Rothera, Journ. of Physiol., **32**; Fischer and Suzuki, Zeitschr. f. physiol. Chem., **45**; Abderhalden, *ibid.*, **51**.

[5] Friedmann, Hofmeister's Beiträge, **3**. With Baer, *ibid.*, 8.

and at the present time we cannot admit of the occurrence of two different cystines.

Cystine probably occurs normally as traces in the urine. In rare cases, in cystinuria, it occurs in larger quantities in the urine sediment or in calculi. Traces have also been found in the ox-kidney, in the liver of the horse and dolphin, and in the liver of a drunkard. ABDERHALDEN[1] has found cystine in the urine and also abundantly in the organs (spleen) in a case of parental cystine diathesis.

The constitution of cystine has been explained by FRIEDMANN,[2] and he has also established the relation between cystine and taurine. Cystine is the disulphide of cysteine, which is α-amino-β-thiolactic acid. From cysteine by oxidation FRIEDMANN obtained cysteinic acid, $C_3H_7NSO_5 = \begin{matrix} CH_2SO_2OH \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{matrix}$, from which taurine $\begin{matrix} CH_2(SO_2OH) \\ \dot{C}H_2(NH_2) \end{matrix}$ is produced by splitting off CO_2.

α-Cystine has also been prepared synthetically. Starting from formyl hippurate, ERLENMEYER, JR. and STOOP first prepared the benzoylserine ester, and then with phosphorus pentasulphide they obtained the benzoylcystine ester. On splitting the latter with HCl they obtained cysteine, and then on oxidation inactive cystine. GABRIEL has also prepared an isocysteine by the cleavage of sulphocyanatedihydrouracil with hydrochloric acid, and then inactive cystine by the oxidation of this isocysteine, and recently FISCHER and RASKE[3] have prepared cystine from α-amino-β-chlorpropionic acid (obtained from *l*-serine) by the action of barium hydrosulphide and a subsequent oxidation in the air.

l-α-Cystine crystallizes in thin, colorless, hexagonal plates. It is not soluble in water, alcohol, ether, or acetic acid, but dissolves in mineral acids and oxalic acid. It is also soluble in alkalies and ammonia, but not in ammonium carbonate. Cystine is optically active, being levorotatory. MÖRNER found it to be $(\alpha)_D = -224.3°$. On heating with hydrochloric acid it can, according to MÖRNER, be changed into a modification crystallizing in needles and with a weaker levorotatory power, or indeed dextrorotatory, composed of a mixture of the two optically active cystines. On heating with HCl to 165° for 12–15 hours NEUBERG and MAYER obtained inactive cystine. By fungus fermentation with Aspergillus niger they obtained dextrorotatory cystine. Cystine has no melting-point but slowly decomposes at 258–261°. On boiling cystine with

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[1] Zeitschr. f. physiol. Chem., 38.

[2] Hofmeister's Beiträge, 3.

[3] Erlenmeyer and Stoop, Ber. d. d. chem. Gesellsch., 36; Gabriel, *ibid.*, 38; Fischer and Raske, *ibid.*, 41.

caustic alkali it decomposes and yields alkali sulphide, which can be detected by lead acetate or sodium nitroprusside. According to MÖRNER [1] 75 per cent of the total sulphur is separated. On treatment of cystine with tin and hydrochloric acid it develops only a little sulphuretted hydrogen, and is converted into cysteine.

On heating upon platinum-foil cystine does not melt, but ignites and burns with a bluish-green flame, with the generation of a peculiar sharp odor. When warmed with nitric acid it dissolves with decomposition, and leaves on evaporation a reddish-brown residue, which does not give the murexid test.

Cystine is gradually precipitated from its sulphuric acid solution by phosphotungstic acid. Cystine forms crystalline salts with mineral acids and with bases. For isolating and separating cystine the precipitation with mercuric acetate is especially suited. The benzoyl cystine (BAUMANN and GOLDMANN [2]) melts at 180–181°; the phenylisocyanate compound at 160°. On boiling with 25 per cent hydrochloric acid this compound passes to the anhydride, which is a hydantoin melting at 119° C.

Stone-cystine, according to NEUBERG and MAYER, differs in many respects from the ordinary cystine, among which the following will be mentioned: The optically active stone-cystine crystallizes in needles, the specific rotation is $(\alpha)_D = -206°$; it melts at 190–192° with marked swelling up. The benzoyl compound melts at 157–159°; the phenylcyanate compound melts at 170–172°, and it is not changed on boiling with hydrochloric acid.

In the detection and identification of cystine we make use of the crystalline form, the behavior on heating on platinum-foil, and the sulphur reaction after boiling with alkali. As to its preparation from protein substances see K. MÖRNER.[3] In regard to the detection of cystine in the urine see Chapter XV.

Cysteine (α-amino-β-thiolactic acid), $C_3H_7NSO_2 = \begin{matrix} CH_2.SH \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$, is formed from cystine by reduction with tin and hydrochloric acid. It is also produced in the cleavage of protein substances, but this is considered by MÖRNER and PATTEN [4] as a secondary formation. Cysteine can be easily converted into cystine by oxidation.

Toward alkalies and lead acetate it acts like cystine. With sodium nitroprusside and alkali it gives a deep purple-red coloration; with ferric chloride the solution gives an indigo-blue coloration which quickly disappears.

Thiolactic acid (α-thiolactic acid) $C_3H_6SO_2 = \begin{matrix} CH_3 \\ | \\ CH(SH) \\ | \\ COOH \end{matrix}$, has been found once as a cleavage product of ox-horn by BAUMANN and SUTER. MÖRNER, FRIEDMANN and BAER obtained it from cystine. It has been shown by FRIED-

[1] Zeitschr. f. physiol. Chem., **34**.
[2] *Ibid.*, **12**.
[3] Zeitschr. f. physiol. Chem., **34**.
[4] See footnote 1, page 79.

MANN that this acid is a regular cleavage product of keratin substances, and that it can also be obtained from the proteins. FRÄNKEL [1] obtained the acid from hæmoglobin. The pyroracemic acid obtained by MÖRNER as a decomposition product from several protein substances originates, according to MÖRNER, only in part from the cystine.

Taurine (aminoethylsulphonic acid), $C_2H_7NSO_3 = \begin{matrix} CH_2.(NH_2) \\ CH_2.(SO_2.OH) \end{matrix}$, has not been obtained as a cleavage product of protein substances; still its origin from proteins has been shown by FRIEDMANN by the close relation that taurine bears to cysteine; and this is the reason why it is treated here in connection with the amino-acids.

Taurine is especially known as a cleavage product of taurocholic acid, and may occur to a slight extent in the intestinal contents. Taurine has also been found in the lungs and kidneys of oxen and in the blood and muscles of cold-blooded animals.

Taurine crystallizes in colorless, often in large, shining, 4- or 6-sided prisms. It dissolves in 15–16 parts of water at ordinary temperatures, but rather more easily in warm water. It is insoluble in absolute alcohol and ether; in cold alcohol it dissolves slightly, but better when warm. Taurine yields acetic and sulphurous acids, but no alkali sulphides, on boiling with strong caustic alkali. The content of sulphur can be determined as sulphuric acid after fusing with saltpeter and soda. Taurine combines with metallic oxides. The combination with mercuric oxide is white, insoluble, and is formed when a solution of taurine is boiled with freshly precipitated mercuric oxide (J. LANG [2]). This compound may be used in detecting the presence of taurine. Taurine is not precipitated by metallic salts.

The preparation of taurine from ox-bile is very simple. The bile is boiled a few hours with hydrochloric acid. The filtrate from the dyslysin and choloidic acid is concentrated well on the water-bath, and filtered hot so as to remove the common salt and other substances which have separated. The solution is evaporated to dryness and the residue dissolved in 5-per cent hydrochloric acid, and precipitated with 10 vols. 95-per cent alcohol. The crystals are readily purified by recrystallization from water.

The alcoholic solution can be used for the preparation of glycocoll. After the evaporation of the alcohol, the residue is dissolved in water, treated with a solution of lead hydroxide, filtered, the lead removed by H_2S, and the filtrate strongly concentrated. The crystals which separate are dissolved and decolorized by animal charcoal and the solution then evaporated to crystallization.

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[1] Mörner, Zeitschr. f. physiol. Chem., **42**; Suter, Zeitschr. f. physiol. Chem., **20**; Friedmann, Hofmeister's Beiträge, **3**; with Baer, *ibid.*, **8**; Fränkel, Sitzungsber. d. Wien. Akad., **112**, II, *b*, 1903.

[2] See Maly's Jahresber., **6**.

Though taurine shows no positive reactions, it is chiefly identified by its crystalline form, by its solubility in water and insolubility in alcohol, by its combination with mercuric oxide, by its non-precipitability by metallic salts, and above all by its sulphur content.

Phenylalanine (phenyl-α-aminopropionic acid), $C_9H_{11}NO_2 = \begin{matrix} C_6H_5 \\ CH_2 \\ CH(NH_2), \\ COOH \end{matrix}$ was first found by E. Schulze and Barbieri [1] in etiolated lupin sprouts. It is produced in the acid cleavage of protein substances in quantities given on pages 106, 107, 114 and 124. It has been prepared synthetically in several ways by Erlenmeyer Jr., Sörensen and E. Fischer.[2]

The *l*-phenylalanine crystallizes in small, shining leaves or fine needles which are rather difficultly soluble in cold water but readily soluble in hot water. The solution has a faint bitter taste. A 5-per cent solution acidified with hydrochloric acid or sulphuric acid is precipitated by phosphotungstic acid, while a more dilute solution is not precipitated. On putrefaction, phenylalanine yields phenylacetic acid. On heating with potassium dichromate and sulphuric acid (25-per cent) an odor of phenylacetaldehyde is produced and benzoic acid is formed. In aqueous solution it has a rotation of $(\alpha)_D = -35.1°$. The phenylisocyanate-phenylalanine melts at about 182° C.

Tyrosine (*p*-oxyphenyl-α-aminopropionic acid), $C_9H_{11}NO_3 = \begin{matrix} C_6H_4(OH) \\ CH_2 \\ CH(NH_2)' \\ COOH \end{matrix}$ is produced from most protein substances under the same conditions as leucine, which it habitually accompanies. The largest quantity of tyrosine obtained from animal proteins was about 10 per cent (see tables, pages 106, 107, 114 and 124). In gelatin and a few keratins tyrosine is absent. It is especially found with leucine, in large quantities, in old cheese (Τυρός), from which it derives its name. Tyrosine has not been found with certainty in perfectly fresh organs. It occurs in the intestine in the digestion of protein substances, and it has about the same physiological and pathological importance as leucine.

Tyrosine was prepared by Erlenmeyer and Lipp from *p*-aminophenylalanine by the action of nitrous acid, and according to another method by Erlenmeyer and Halsey.[3] On fusing with caustic alkali

[1] Ber. d. d. chem. Gesellsch., **14**, and Zeitschr. f. physiol. Chem., **12**.

[2] Erlenmeyer, Annal. d. Chem. u. Pharm., **275**; Sörensen, Zeitschr. f. physiol. Chem., **44**; E. Fischer, Ber. d. d. chem. Gesellsch., **37**.

[3] Erlenmeyer and Lipp, Ber. d. d. chem. Gesellsch., **15**; Erlenmeyer and Halsey, *ibid.*, **30**.

it yields *p*-oxybenzoic acid, acetic acid, and ammonia. On putrefaction it may yield *p*-hydrocoumaric acid, oxyphenylacetic acid, and *p*-cresol.

Naturally occurring tyrosine and that obtained by the cleavage of protein substances by acids or enzymes, is generally *l*-tyrosine, while that obtained by decomposition with baryta-water or prepared synthetically is inactive. v. LIPPMANN [1] has obtained *d*-tyrosine from beet-sprouts. The statements as to specific rotation of tyrosine are somewhat variable. For tyrosine from proteins E. FISCHER has found a rotation of $(\alpha)_D = -12.56$ to 13.2° for the hydrochloric acid solution, while SCHULZE and WINTERSTEIN [2] obtained higher results using tyrosine from plants, namely, $(\alpha)_D = -16.2°$. These investigators believe that when lower results are obtained a contamination with racemic tyrosine is the cause.

Tyrosine in a very impure state occurs in the form of balls similar to leucine. The purified tyrosine, on the contrary, appears as colorless, silky, fine needles which are often grouped into tufts or balls. It is soluble with difficulty in water, being dissolved by 2454 parts water at 20° C., and 154 parts boiling water, separating, however, as tufts of needles on cooling. It dissolves more easily in the presence of alkalies, ammonia, or a mineral acid. It is difficultly soluble in acetic acid. Crystals of tyrosine separate from an ammoniacal solution on the spontaneous evaporation of the ammonia. One hundred parts glacial acetic acid dissolve on boiling only 0.18 part tyrosine, and by this means, especially on adding an equal volume of alcohol before boiling, the leucine can be quantitatively separated from the tyrosine (HABERMANN and EHRENFELD [3]). The *l*-tyrosine-ethyl-ester crystallizes in colorless prisms which melt at 108–109°C. The naphthylisocyanate-*l*-tyrosine melts at 205–206°. Tyrosine can be oxidized with the formation of dark-colored products by various plant as well as animal oxidases, so-called tyrosinases (see Chapter I). Tyrosin is identified by its crystalline form and by the following reactions:

PIRIA'S *Test* Tyrosine is dissolved in concentrated sulphuric acid by the aid of heat, by which tyrosine-sulphuric acid is formed; it is allowed to cool, diluted with water, neutralized by $BaCO_3$, and filtered. On the addition of a solution of ferric chloride the filtrate gives a beautiful violet color. This reaction is disturbed by the presence of free mineral acids and by the addition of too much ferric chloride.

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[1] Ber. d. d. chem. Gesellsch., **17**.

[2] See Hoppe-Seyler-Thierfelder, Handb. d. physiol. u. pathol. chem. Analyse, 8. Aufl., 1909. Also E. Fischer, Ber. d. d. chem. Gesellsch., **32**; Schulze and Winterstein, Zeitschr. f. physiol. Chem., **45**.

[3] Zeitschr. f. physiol. Chem., **37**.

HOFMANN'S *Test.* If some water is poured on a small quantity of tyrosine in a test-tube and a few drops of MILLON'S reagent added and then the mixture boiled for some time, the liquid becomes a beautiful red and then yields a red precipitate. Mercuric nitrate may first be added, then, after this has boiled, nitric acid containing some nitrous acid.

DENIGÈS' *Test,* modified by C. MÖRNER,[1] is performed as follows: To a few cubic centimeters of a solution consisting of 1 vol. formaline, 45 vols. water, and 55 vols. concentrated sulphuric acid add a little tyrosine in substance or in solution and heat to boiling. A beautiful permanent green coloration is obtained.

Proline (α-pyrolidine carboxylic acid), $C_5H_9NO_2 = \begin{array}{l} H_2C—CH_2 \\ \quad | \qquad\quad | \\ H_2C \quad CH.COOH, \\ \quad \diagdown \quad \diagup \\ \qquad NH \end{array}$

was first obtained by E. FISCHER and then by FISCHER and collaborators[2] from several proteins. The proline here obtained was generally the levo-rotatory modification. The largest quantity of proline was secured from the vegetable protein hordein, namely, 13.7 per cent, and also from the egg membrane of the Testudo græca, 11.8 per cent (see table pages 106, 107, 114 and 124). KOSSEL and DAKIN[3] got 11 per cent from salmine. Proline also occurs in scombrine and clupeine, but not in sturine, which, according to KOSSEL, seems to contradict the view as to the common origin of ornithine and proline.

SÖRENSEN,[4] by means of a general method of preparing α-amino-acids synthetically, has prepared α-amino-δ-oxyvaleric acid from phthalimide-malonic ester and has obtained proline from this by evaporating with hydrochloric acid, at the same time splitting off water. Recently he has suggested another method which yields good results. Other syntheses of proline have also been performed by E. FISCHER and WILLSTÄDTER.[5]

l-Proline crystallizes in flat needles. It is readily soluble in water and alcohol. The solution has a sweet taste; the specific rotation at 20° C. is $(\alpha)_D = -77.40°$. The solution acidified with sulphuric acid is precipitated by phosphotungstic acid. In the detection of this acid we make use of the copper salt, the anhydride of the phenylisocyanate compound (melting-point 144°), and the picrate (ALEXANDROFF[6]).

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[1] Denigès, Compt. rend., **130**; C. Th. Mörner, Zeitschr. f. physiol. Chem., **37**.

[2] E. Fischer, Zeitschr. f. physiol. Chem., **33** and **35**. See also footnote 2, page 85.

[3] Zeitschr. f. physiol. Chem., **41**.

[4] Zeitschr. f. physiol. Chem., **44**; with A. C. Anderson, *ibid.*, **56**.

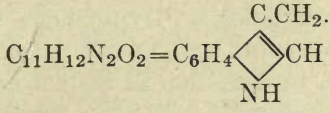
[5] Ber. d. d. chem., Gesellsch., **33**.

[6] Zeitschr. f. physiol. Chem., **46**.

The inactive acid and its compounds show somewhat varying properties.

Oxyproline (oxy-α-pyrolidine carboxylic acid), $C_5H_9NO_3$. This acid, whose constitution is not understood was first obtained by E. FISCHER[1] on the hydrolysis of casein and of gelatin. It dissolves readily in water; has a specific rotation of $(\alpha)_D = -81.04°$, and the solution has a sweet taste. Oxyproline crystallizes in beautiful colorless plates and gives a readily soluble copper salt. LEUCHS[2] has prepared two isomeric inactive oxyprolines which were crystalline.

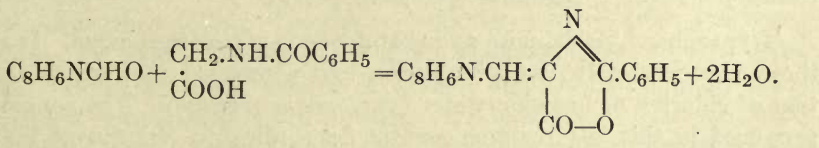
Tryptophane (indolaminopropionic acid),



is one of the cleavage products of the proteins formed in tryptic digestion and other deep decompositions of the proteins, such as putrefaction, cleavage with baryta-water or sulphuric acid. It gives a reddish-violet product with chlorine or bromine which is called *proteinochrome*. NENCKI[3] considered tryptophane, which name is generally given to this acid, as the mother-substance of various animal pigments.

Tryptophane was first prepared in a pure form by HOPKINS and COLE,[4] and they considered it as skatolaminoacetic acid. After ELLINGER showed that skatolcarbonic acid (SALKOWSKI) and skatolacetic acid (NENCKI) were indolacetic acid and indolpropionic acid respectively, and after the synthesis of *d-l*-tryptophane by ELLINGER and FLAMAND,[5] the nature of this substance as indolaminopropionic acid was established.

By condensation of β-indolaldehyde with hippuric acid ELLINGER and FLAMAND prepared the azlactone (lactimide):



[1] Ber. d. d. chem. Gesellsch., **35** and **36**.

[2] Ber. d. d. chem., Gesellsch., **38**. See also Leuchs and Felser *ibid.*, **41**.

[3] In regard to tryptophane, see Stadelmann, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **26**; Nencki, Ber. d. d. chem. Gesellsch., **28**; Beitler, *ibid.*, **31**; Kurajeff, Zeitschr. f. physiol. Chem., **26**; Klug, Pflüger's Arch., **86**.

[4] Journ. of Physiol., **27**.

[5] Ellinger, Ber. d. d. Chem. Gesellsch., **37** and **38**. With Flamand, *ibid.*, **40**, and Zeitschr. f. physiol. Chem., **55**.

On boiling with dilute caustic soda, with the taking up of water, the sodium salt of indoxyl-α-benzoylaminoacrylic acid,

$$C_8H_6N.\,CH{:}\underset{\dot{C}OONa}{C}.NH.COC_6H_5$$

is obtained, from which by reduction and splitting off of the benzoyl group by the action of sodium alcoholate the tryptophane is obtained:

$$\begin{array}{l} C_8H_6N.CH{:}\,C.NH.COC_6H_5 \\ \qquad\qquad\quad | \\ \qquad\qquad\quad CCOH \end{array} + H_2 + H_2O = C_8H_6N.CH_2.\underset{\dot{C}OOH}{CH}.NH_2 + C_6H_5COOH.$$

The trytophane formed in digestion is *l*-tryptophane, which is levorotatory in aqueous solution (HOPKINS and COLE). Racemic *d-l*-tryptophane has also been obtained by digestion in certain cases by ALLERS and NEUBERG; this is probably formed from the *l*-tryptophane (ABDERHALDEN and L. BAUMANN [1]), which very readily undergoes racemization.

Tryptophane crystallizes in silky, rhombic or six-sided leaves. It does not have a sharp melting-point, and according to the rapidity of heating melts at 252°, 273° and 289°, according to various authorities. Tryptophane is readily soluble in hot water, difficultly soluble in cold water, and only slightly soluble in alcohol. The solution of *d-l*-tryptophane has a faint sweetish taste, and *l*-tryptophane a faint bitter taste. The statements as to the optical behavior of tryptophane differ somewhat (HOPKINS and COLE, NEUBERG and POPOWSKY, ABDERHALDEN and KEMPE, ELLINGER and FLAMAND, H. FISCHER [2]) which, according to ABDERHALDEN, is probably due to the readiness with which it undergoes racemization. According to ABDERHALDEN and L. BAUMANN, at 20° C. the aqueous solution has a rotation of $(\alpha)_D = -30.33°$. HOPKINS and COLE give $(\alpha)_D = -33°$ for the watery solution. It is dextro rotatory in $\frac{N}{1}$ or $\frac{N}{2}$ NaOH as well as in $\frac{N}{1}$ HCl.

Tryptophane yields indol and skatol when sufficiently heated. It gives the ADAMKIEWICZ-HOPKINS [3] reaction and a rose-red color on the addition of chlorine or bromine water (*tryptophane reaction*). The compound produced in this last reaction contains, according to NEUBERG,[4] 1 atom of bromine (or chlorine) while another compound containing 3 halogen atoms is yellow in color. If a pine stick previously moistened

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[1] R. Allers, Biochem. Zeitschr., 6; C. Neuberg, *ibid.*, 6; Abderhalden and Baumann, Zeitschr. f. physiol. Chem., 55. (Literature on the specific rotation.)

[2] See Abderhalden and Baumann, Zeitschr. f. physiol. Chem., 55 (literature).

[3] In regard to this reaction see also Dakin, Journ. of Biol. Chem. 2, and O. Rosenheim, Biochem. Journ., 1.

[4] Biochem. Zeitschr., 2 and 6. See also Levene and Rouiller, *ibid.*, 4.

with hydrochloric acid and washed with water is introduced into a concentrated tryptophane solution, it becomes purple (pyrrole reaction) on drying. The melting-points of the benzoylsulphotryptophane, the β-naphthalenesulphotryptophane and the naphthylisocyanatetryptophane are according to ELLINGER and FLAMAND,[1] 185°, 180° and 158° C. respectively. Several compounds of tryptophane have been prepared by ABDERHALDEN and KEMPE.[2] Among these we will mention the tryptophane chloride hydrochloride, because it is used as the starting material for the synthesis of tryptophane polypeptides.

In regard to the rather complicated method for preparing tryptophane we must refer to the original work of HOPKINS and COLE, of NEUBERG, and of ABDERHALDEN and KEMPE.[3]

As shown by HOPKINS and COLE,[4] tryptophane on anaerobic putrefaction yields indolpropionic acid and indolacetic acid, and indol and skatol on aerobic putrefaction. Among these putrefactive products the indol and skatol will be specially discussed.

Indol, $C_8H_7N=C_6H_4\langle{}^{CH}_{NH}\rangle CH$, and **Skatol,** or β-METHYLINDOL, $C_9H_9N=C_6H_4\langle{}^{C.CH_3}_{NH}\rangle CH$, are formed in variable quantities from protein compounds under different conditions. Hence they occur habitually in the human intestinal canal, and, after oxidation into indoxyl and skatoxyl respectively, pass, at least partly, into the urine as the corresponding ethereal sulphuric acids and also as glucuronic acids.

Indol and skatol crystallize in shining leaves, and their melting-points are 52° and 95° C. respectively. Indol has a peculiar excrementitious odor, while skatol has an intense fetid odor (skatol obtained from indigo is odorless). Both bodies are easily volatilized by steam, skatol more easily than indol. They may both be removed from the watery distillate by ether. Skatol is the more insoluble of the two in boiling water. Both are easily soluble in alcohol and give with picric acid a compound crystallizing in red needles. If a mixture of the two picrates be distilled with ammonia, they both pass over without decomposition: while if they are distilled with caustic soda, the indol but not the skatol is decomposed. The watery solution of indol gives with fuming nitric

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[1] l. c.

[2] Zeitschr. f. physiol. Chem., 52, and Ber. d. d. chem. Gesellsch., 40.

[3] Hopkins and Cole, Journ. of Physiol., 27 and 29; Neuberg and Popowsky, Biochem. Zeitschr., 2; Abderhalden and Kempe, Zeitschr. f. physiol. Chem., 52.

[4] Journ. of Physiol., 29.

acid a red liquid and then a red precipitate of nitroso-indol nitrate (NENCKI [1]). It is better first to add two or three drops of nitric acid and then a 2-per cent solution of potassium nitrite, drop by drop (SALKOWSKI [2]). Skatol does not give this reaction. An alcoholic solution of indol treated with hydrochloric acid colors a pine chip cherry-red. Skatol does not give this reaction. Indol gives a deep reddish-violet color with sodium nitroprusside and alkali (LEGAL'S reaction). On acidifying with hydrochloric acid or acetic acid the color becomes pure blue. Skatol does not act the same. The alkaline solution is yellow and becomes violet on acidifying with acetic acid and boiling. With a few drops of a 4-per cent formaline solution and concentrated sulphuric acid indol gives a beautiful violet color while skatol gives a yellow or brown color (KONDO [3]). Skatol dissolves in concentrated hydrochloric acid with a violet coloration. On warming skatol with sulphuric acid a beautiful purple-red coloration is obtained (CIAMICIAN and MAGNANINI [4]).

For the detection of indol and skatol in, and their preparation from, feces and putrefying mixtures, the main points of the usual method are as follows: The mixture is distilled after acidifying with acetic acid; the distillate is then treated with alkali (to combine with any phenols which may be present) and again distilled. From this second distillate the two bodies, after the addition of hydrochloric acid, are precipitated by picric acid. The precipitated picrate is then distilled with ammonia. The two bodies are obtained from the distillate by repeated shaking with ether and evaporation of the several ethereal extracts. The residue, containing indol and skatol, is dissolved in a very small quantity of absolute alcohol and treated with 8–10 vols. of water. Skatol is precipitated, but not the indol. The further treatment necessary for their separation and purification will be found in other works.[5]

Skatosine, $C_{10}H_{16}N_2O_2$, is a base first obtained by BAUM in the pancreas autodigestion and later studied by SWAIN. It develops an indol- or skatol-like odor on fusing with potassium hydroxide. LANGSTEIN [6] obtained a substance which is perhaps identical with skatosine, in the very lengthy peptic digestion of blood proteins.

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[1] Ber. d. d. deutsch. chem. Gesellsch., 8, 727, and *ibid.*, 722 and 1517.

[2] Zeitschr. f. physiol. Chem., 8, 447. In regard to newer reactions for indol and skatol, see Steensma, *ibid.*, 47, and Denigès, Compt. rend. soc. biol., 64.

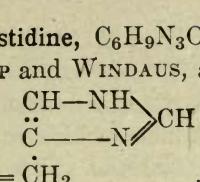
[3] Zeitschr. f. physiol. Chem., 48; see also Dakin, Journ. of biol. Chem., 2.

[4] Ber. d. d. chem. Gesellsch., 21, 1928.

[5] For quantitative, colorimetric determinations of indol in feces see Einhorn and Hübner, Salkowski's Festschrift, Berlin, 1904; C. A. Herter and Foster, Journ. of biol. Chem., 2.

[6] Baum, Hofmeister's Beiträge, 3; Swain, *ibid.*; Langstein, see Hofmeister, Ueber Bau und Gruppierung der Eiweisskörper, in Ergebnisse der Physiologie, I, Abt. 1, 1902.

Histidine, $C_6H_9N_3O_2$, according to the investigations of H. PAULY, KNOOP and WINDAUS, and **F. KNOOP**[1], is an α-amino-β-imidazolpropionic acid,



Histidine was first discovered by KOSSEL in the cleavage products of sturine. It was then found by HEDIN in the cleavage products of proteins by acid hydrolysis, and by KUTSCHER among the products of tryptic digestion, and finally also as a cleavage product of different protein substances. It does not occur in the protamines, with the exception of sturine. Of the protein bodies globin (from horse-hæmoglobin) seems to be richest in histidine, as ABDERHALDEN found 10.96 per cent. It also occurs in germinating plants (E. SCHULZE[2]).

Histidine crystallizes in colorless needles and plates and is readily soluble in water, but less soluble in alcohol, and has an alkaline reaction. It is precipitated by phosphotungstic acid, but this precipitate is soluble in an excess of the precipitant (FRÄNKEL). With silver nitrate alone the aqueous solution is not precipitated; on the careful addition of ammonia or baryta-water an amorphous precipitate, which is readily soluble in an excess of ammonia, is obtained. Histidine can be precipitated by mercuric chloride, or, still better, by the sulphate acidified with sulphuric acid, and can in this way be separated from the other diamino-acids (KOSSEL and PATTEN). The hydrochloride crystallizes in beautiful plates (BAUER), dissolves rather readily in water, but is insoluble in alcohol and ether. With hydrochloric acid and methyl alcohol it gives the dihydrochloride of histidine methyl ester, which melts at 196.° Histidine is levorotatory, $(\alpha)_D = -39.74°$, while its solution in hydrochloric acid is dextrorotatory. On warming it gives the biuret test (HERZOG), and it also gives WEIDEL'S reaction if performed as suggested by FISCHER (see Xanthine, Chapter V) (FRÄNKEL[3]). On adding sufficient bromine water and warming, a reddish coloration ensues

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[1] Pauly, Zeitschr. f. physiol. Chem., **42**; Knoop and Windaus, Hofmeister's Beiträge, **7** and **8**; Knoop, *ibid.*, **10**.

[2] Kossel, Zeitschr. f. physiol. Chem., **22**; Hedin, *ibid.*, Kutscher, *ibid.*, **25**; Wetzel, *ibid.*, **26**; Lawrow, *ibid.*, **28**, and Ber. d. d. chem. Gesellsch., **34**; Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**; Hart, *ibid.*, **33**; Abderhalden, *ibid.*, **37**; Schulze, *ibid.*, **24** and **28**.

[3] Kossel and Patten, Zeitschr. f. physiol. Chem., **38**; Bauer, *ibid.*, **22**; Herzog, *ibid.*, **37**; Fränkel, Sitz.-Ber. d. Wien. Akad., **112**, II. B., 1903, and Hofmeister's Beiträge, **8**.

which turns deep wine-red, later becoming cloudy, due to the formation of dark amorphous particles (F. KNOOP [1]).

It gives a very beautiful diazo-reaction with diazobenzenesulphonic acid in solutions made alkaline with sodium carbonate, which according to PAULY is deep cherry-red in dilutions of 1:20,000 and still markedly red in 1:100,000 (tyrosine gives a similar reaction).

Histidine is sometimes classified in a group, with the two diamino-acids, arginine and lysine which KOSSEL has called the *hexone bases.*

Arginine (guanidine-α-aminovaleric acid),

$$C_6H_{14}N_4O_2 = \begin{matrix} (HN)C<^{NH_2}_{NH.CH_2} \\ (CH_2)_2 \\ CH(NH_2) \\ COOH \end{matrix},$$

first discovered by SCHULZE and STEIGER in etiolated lupin- and pumpkin-sprouts, has later been found in other germinating plants, in tubers and roots. GULEWITSCH has found arginine in the ox-spleen. It was first found by HEDIN as a cleavage product of horn substance, gelatin, and several proteins, and then by KOSSEL and his pupils as a general cleavage product of protein substances as a class. The greatest quantity was obtained from the protamines; but the histones and certain plant proteins, edestin and the protein from pine seeds and especially excelsin (14.14 per cent), also yield abundant arginine. Arginine also occurs among the products of tryptic digestion (KOSSEL and KUTSCHER [2]).

On boiling with baryta-water, as well as by the action of an enzyme, *arginase,* discovered by KOSSEL and DAKIN,[3] arginine yields urea and ornithine. Arginine has been prepared synthetically from ornithine (α-δ-diamino-valeric acid) and cyanamide by SCHULZE and WINTERSTEIN.[4]

Arginine crystallizes in rosette-like tufts, plates, or thin prisms, is readily soluble in water with alkaline reaction and nearly insoluble in alcohol. With several acids and metallic salts it forms crystalline salts and double salts respectively. Its acidified watery solution is precipitated by phosphotungstic acid. The most important salts are the copper-nitrate $(C_6H_{14}N_4O_2)_2.Cu(NO_3)_2+3H_2O$ and the silver salts $C_6H_{14}N_4O_2.HNO_3+$

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[1] Hofmeister's Beitrage, **11**

[2] Schulze and Steiger, Zeitschr. f. physiol. Chem., **11**; Schulze and Castoro, *ibid.*, **41**; Gulewitsch, *ibid.*, **30**; Hedin, *ibid.*, **20** and **21**; Kossel and Kutscher, *ibid.*, **22**, **25**, **26**.

[3] Zeitschr. f. physiol. Chem., **41**, and Dakin, Journ. of biol. Chem., **3**.

[4] Ber. d. d. chem. Gesellsch., **32** and Zeitschr. f. physiol. Chem., **34**.

$AgNO_3$ (the more readily soluble) and $C_6H_{14}N_4O_2.AgNO_3+\frac{1}{2}H_2O$ (the more difficultly soluble), and its compound with picrolonic acid (STEUDEL[1]).

Arginine is dextrorotatory. In 9–10 per cent solution in the presence of 1 mol. HCl and at 20° C., it has a specific rotation $(\alpha)_D=+10.7°$. The arginine obtained by KUTSCHER in the tryptic digestion of fibrin was racemic arginine. KUTSCHER has prepared this racemic arginine from *d*-arginine, and RIESSER[2] has obtained *l*-arginine by splitting racemic arginine with arginase. On oxidation with permanganate it splits off guanidine, which can be precipitated with sodium picrate. ORGLMEISTER[3] bases his method for the quantitative estimation of arginine in mixtures obtained by hydrolysis upon this fact.

Ornithine (α-δ-diaminovaleric acid), $C_5H_{12}N_2O_2=\begin{matrix}CH_2.(NH_2)\\(CH_2)_2\\CH(NH_2)\\COOH\end{matrix}$, is not a primary cleavage product of proteins, but is formed from arginine on boiling with baryta-water. JAFFE,[4] who first discovered this body, obtained it as a cleavage product from ornithuric acid, which is found in the urine of hens fed with benzoic acid. The ornithine which E. FISCHER and recently SÖRENSEN[5] have prepared synthetically yields, as shown by ELLINGER, putrescine (tetramethylenediamine), $C_4H_8(NH_2)_2$, on putrefaction. A. LOEWY and NEUBERG[6] have shown that ornithine is split into putrescine and CO_2 in the organism of cystinuria patients.

Ornithine is a non-crystalline substance which dissolves in water, giving an alkaline reaction, and yields several crystalline salts. It is precipitated by phosphotungstic acid and several metallic salts, but not by silver nitrate and baryta-water (differing from arginine). Ornithine hydrochloride is dextrorotatory; the synthetically prepared one is inactive. On shaking ornithine with benzoyl chloride and caustic soda it is converted into dibenzoylornithine (ornithuric acid). On splitting artificially prepared racemic ornithuric acid SÖRENSEN has shown that the naturally occurring ornithuric acid is identical with the dextrorotatory α-δ-dibenzoyldiaminovaleric acid.

Diaminoacetic acid, $C_2H_6N_2O_2=CH(NH_2)_2COOH$ was obtained by DRECHSEL[7] as a cleavage product of casein by boiling with tin and hydrochloric acid. It crystallizes in prisms and gives a monobenzoyl compound which is not very soluble in cold water and is nearly insoluble in alcohol, and can be used in the isolation of the acid.

Lysine (α-ε-diaminocaproic acid), $C_6H_{14}N_2O_2=\begin{matrix}CH_2(NH_2)\\(CH_2)_3\\CH(NH_2)\\COOH\end{matrix}$, was first

[1] Zeitschr. f. physiol. Chem., **37** and **44**.
[2] Kutscher, Zeitschr. f. physiol. Chem., **28** and **32**; Riesser, *ibid.*, **49**.
[3] Hofmeister's Beiträge, **7**.
[4] Ber. d. d. chem. Gesellsch., **10** and **11**.
[5] Fischer, *ibid.*, **34**; Sörensen, Zeitschr. f. pyhsiol. Chem., **44**.
[6] Ellinger, Zeitschr. f. physiol. Chem., **29**; Loewy and Neuberg, *ibid.*, **43**.
[7] Ber. d. sächs. Ges. d. Wissensch., **44**.

obtained by DRECHSEL as a cleavage product of casein. Later he and his pupils, as well as KOSSEL and others, found it among the cleavage products of various proteins. It has not been detected in some vegetable proteins such as zein and gluten-protein. E. SCHULZE found lysine in germinating plants of the Lupinus luteus, and WINTERSTEIN found it in ripe cheese. It has been obtained in largest amounts (28.8 per cent) by KOSSEL and DAKIN[1] from the protamine α-cyprinine.

Lysine has been synthetically prepared by E. FISCHER and WEIGERT.[2] This lysine was inactive, while that prepared from protein is always optically active and dextrorotatory. The rotation depends upon the concentration and degree of acidity; for the hydrochloride a rotation of $(\alpha)_D = +14°$ to 17.25° has been found. On heating with barium hydroxide it is converted into the inactive modification. According to ELLINGER[3] lysine yields cadaverine (pentamethylenediamine), $C_5H_{10}(NH_2)_2$, on putrefaction, and this base is formed from the lysine in the organism of those with cystinuria and at the same time CO_2 is split off (A. LOEWY and NEUBERG).

Lysine is readily soluble in water but is not crystallizable. The aqueous solution is precipitated by phosphotungstic acid, but not by silver nitrate and baryta-water (differing from arginine and histidine). It gives two hydrochlorides with hydrochloric acid, and with platinum chloride a chloroplatinate which is precipitable by alcohol and has the composition $C_6H_{14}N_2O_2.H_2PtCl_6 + C_2H_5OH$. It gives two silver salts with $AgNO_3$; one has the formula $AgNO_3 + C_6H_{14}N_2O_2$ and the other $AgNO_3 + C_6H_{14}N_2O_2.HNO_3$. With benzoyl chloride and alkali, lysine forms an acid, *lysuric acid*, $C_6H_{12}(C_7H_5O)_2N_2O_2$ (DRECHSEL), which is homologous with ornithuric acid, and whose difficultly soluble acid barium salt may be used in the separation of lysine.[4] The rather insoluble picrate, which is precipitated from a not too dilute solution of the hydrochloride by sodium picrate, may also be used in the detection of lysine.

KUTSCHER and LOHMANN[5] have found a lysine having somewhat different properties in the final products of pancreas autolysis.

Lysatine or Lysatinine. The formula of this substance is either $C_6H_{13}N_3O_2$ or

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[1] Drechsel, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., **25**; Siegfried, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., **24**; Hedin, Zeitschr. f. physiol. Chem., **21**; Kossel, *ibid.*, **25**; Kossel and Mathews, *ibid.*, **25**; Kossel and Kutscher, *ibid.*, **31**; Kutscher, *ibid.*, **29**; Schulze, *ibid.*, **28**; Winterstein, cited in Schulze and Winterstein, Ergebnisse der Physiologie, I, Abt. 1, 1902; Kossel and Dakin, Zeitschr. f. physiol. Chem., **40**.

[2] Ber. d. d. chem. Gesellsch., **35**.

[3] Zeitschr. f. physiol. Chem., **29**.

[4] Drechsel, Ber. d. d. chem. Gesellsch., **28**; see also C. Willdenow, Zeitschr. f. physiol. Chem., **25**.

[5] Zeitschr. f. physiol. Chem., **41**.

$C_6H_{11}N_3O+H_2O$. In the first case this base would appear to be a homologue of creatine, $C_4H_9N_3O_2$, and in the other case a homologue of creatinine, $C_4H_7N_3O$, and this is the reason why this body is called lysatine as well as lysatinine. It is still a question whether lysatine is a chemical individual or, as HEDIN suggests, only a mixture of lysine and arginine.[1]

In the preparation of the above bases we can first precipitate all the bases by phosphotungstic acid, when the monamino-acids remain in solution. The precipitate is then decomposed in boiling water by barium hydroxide and the bases obtained as silver compounds from this filtrate. In regard to further details we must refer to the works of DRECHSEL and HEDIN already cited. KOSSEL and KUTSCHER and recently WINTERSTEIN [2] have suggested a method of separating histidine and arginine as silver compounds from lysine, and KOSSEL and PATTEN have proposed a method of separating histidine from arginine by means of mercuric sulphate.

We give below a tabulation of the amounts of the three hexone bases found in certain protein substances (in weight per cent):

	Arginine.	Lysine.	Histidine.
Sturine [3]	58.2	12.0	12.9
Cyprinine (α) [7]	4.9	28.8	0.0
Other protamines [3]	62.5—87.4	0.0	0.0
Histones [3]	14.36—15.52	7.7—8.3	1.21—2.34
Casein [4]	4.70—4.84	1.92—5.80	2.53—2.59
Syntonin (from meat) [4]	5.06	3.26	2.66
Heterosyntonose [4]	8.53	3.08—7.03	0.37—1.12
Protosyntonose [4]	4.55	3.08	3.35
Edestin [5]	11.0—14.07	1.3	1.17
Proteid from coniferæ seeds [5]	10.9—11.3	0.25—0.79	0.62—0.78
Gluten casein [3]	4.4	2.15	1.16
Gluten proteins [3]	2.75—3.13	0.0	0.43—1.53
Gelatin [3] and [4]	7.62—9.3	2.49—6.0	0.40
Elastin [6]	0.3	+	0.027

Of the oxydiamino-acids found on the hydrolysis of proteins we will mention the following:

Oxydiaminosebacic acid, (?)$C_{10}H_{20}N_2O_5$, has been isolated by WOHLGEMUTH from a nucleoprotein of the liver. The free acid was obtained as small white plates. It is soluble with difficulty in hot water, insoluble in cold water and in alcohol. It was optically inactive in hydrochloric acid. The beautifully crystalline phenylcyanate compound had a melting-point of 206°.

Dioxydiaminosuberic acid, $C_8H_{16}N_2O_6$, has been obtained by SKRAUP [9] on the hydrolysis of casein with hydrochloric acid. The copper salt crystallizes in

[1] Hedin, Zeitschr. f. physiol. chem., **21**.

[2] Kossel and Kutscher, *ibid.*, **31**; Winterstein, *ibid.*, **45**; Kossel and Patten, l. c.

[3] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**.

[4] Hart, *ibid.*, **33**.

[5] Schulze and Winterstein, *ibid.*, **33**; see also Kossel, Ber. d. d. chem. Gesellsch., **34**, 3236.

[6] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **25**, and Richards and Gies, Amer. Journ. of Physiol., **7**.

[7] Kossel and Dakin, Zeitschr. f. physiol. Chem., **40**.

[8] Ber. d. d. chem. Gesellsch., **37**, and Zeitschr. f. physiol. chem. **44**.

[9] Zeitschr. f. physiol. Chem., **42**.

beautiful deep bluish-violet rosettes which are composed of long, irregular, right-angled plates. It is quite soluble in cold water. The free acid crystallizes in fern-like formations. Besides this acid SKRAUP obtained two other acids which he calls *caseanic acid*, $C_9H_{16}N_2O_7$, and *caseinic acid*, $C_{12}H_{24}N_2O_5$. The caseanic acid crystallizes, melts at 190–191°, is tribasic, and is probably an oxydiamino-acid. The caseinic acid is dibasic and occurs in two modifications. The one which melts at 228° is faintly dextrorotatory; the other modification melts at 245° and is optically inactive. Both crystallize, but the inactive form does not yield well-defined crystals. Caseinic acid seems also to be an oxydiamino-acid.

Diaminotrioxydodecanoic acid, $C_{12}H_{26}N_2O_5$, is an acid obtained by FISCHER and ABDERHALDEN [1] on the hydrolysis of casein and seems to stand close to SKRAUP'S caseinic acid, but differs from it in its optical properties. This acid is faintly levorotatory: $(\alpha)_D$ = about −9°. It crystallizes in plates, which grow into rosettes or spherical aggregations. It has a faint bitter taste, gives a crystalline hydrochloride which is slightly soluble in strong hydrochloric acid, and gives a crystalline copper salt.

In regard to the methods which are used in the separation and preparation of the amino-acids and other products produced in the hydrolysis of proteins, we refer the reader to larger handbooks and to E. FISCHER'S work, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Berlin, 1906.[2]

In order to quantitatively follow the proteolysis, especially the later stages, one can, according to SÖRENSEN,[3] determine to advantage the increase in the carboxyl groups which takes place during the progress of the hydrolysis. For this purpose he has worked out a method which is as follows: The amino groups are converted into methylene groups by means of formaldehyde and then the carboxyl groups determined acidimetrically by $\frac{N}{5}Ba(OH)_2$ (or in certain cases by caustic alkali) using phenolphthalein or thymolphthalein as indicator. By this method not only can enzymotic products be titrated, but also products of acid hydrolysis after decolorization by means of silver nitrate.

SIEGFRIED [4] has found that amino-acids in the presence of alkali or alkaline earths de-ionize carbon dioxide and form salts of the type of the carbamino salts. For example glycocoll in the presence of lime yields with carbon dioxide, calcium carbamino-acetic acid,

$$\begin{array}{l} CH_2.NH.COO \\ \;\;| \qquad\qquad\;\; | \\ COO\text{————}Ca \end{array}.$$

If the nitrogen is determined and at the same time the combined carbon dioxide estimated by means of the calcium carbonate split off on boiling the filtered solution, then the quotient $\frac{CO_2}{N}$ gives the number of N atoms

[1] Zeitschr. f. physiol. Chem., 42.

[2] See Hoppe Seyler-Thierfelder, Handb. d. physiol. in pathol. Chem. Analyse, 8 Aufl., Berlin, 1909, and also Abderhalden in Handb. der Biochem. by C. Oppenheimer, Bd. 1, p. 347.

[3] Biochem. Zeitschr., 7; with Hansen, *ibid.*, 7.

[4] Zeitschr. f. physiol. Chem., 44 and 46; with Neumann, *ibid.*, 54; with Liebermann, *ibid.*, 54.

for every molecule CO_2 taken up. This quotient is equal to 1 for glycocoll and the aliphatic amino-acids because these go over quantitatively into carbamino-acids. With the diamino-acid arginine, which contains 4 nitrogen atoms, it is on the contrary only one-fourth because this acid reacts with only one amino group, that of the α-amino valeric acid chain.

This behavior, which has been further studied by SIEGFRIED with NEUMANN and LIEBERMANN, is valuable in determining whether we are dealing with a mixture of protein cleavage products or a combination of these, as the quotient becomes larger by the splitting of a peptide binding. As the quotient also increases during the progress of digestion SIEGFRIED and his collaborators have attempted to control the progress of proteolytic cleavage by determining this quotient.

II. Compound Proteins [1]

We designate as compound proteins those bodies which yield, on cleavage, proteins (with their decomposition products) and other bodies such as carbohydrates, nucleic acids, or pigments.

The compound proteins known at present can be divided into three groups: *glycoproteins*, *nucleoproteins* and *chromoproteins*. Of these the last-mentioned group (hæmoglobin and hæmocyanine) will be discussed in a subsequent chapter (Chapter VI on the blood).

a. Glycoproteins (glucoproteins).

Glycoproteins [2] are those compound proteins which on decomposition yield a protein on the one side, and a carbohydrate or derivatives of this on the other, but no purine bodies. Some glycoproteids are free from phosphorus (mucin substances, chondroproteins, and hyalogens), and some contain phosphorus (phosphoglycoproteins).

The glycoproteins free from phosphorus may, as regards the nature of the carbohydrate groups split off, be divided into two chief groups, the *mucin substances* and the *chondroproteins*. The first yield on hydrolytic

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[1] Hoppe-Seyler has given the name *proteïde* to these compound proteids, but as this term is misleading in English we do not use it in English classifications in this sense.

[2] Abderhalden (Lehrb. d. physiol. Chem., 1909, p. 191) has proposed dropping the name glycoproteids entirely and to consider these bodies as simple proteins, because it has not been shown that the carbohydrate groups occupy the same relationship to the protein component that the hæmin or the nucleic acid bears to the hæmoglobin or the nucleoprotein molecule. It is possible that this proposition, which is not applicable to the entire group (including the proteins containing chondroitin-sulphuric acid) but applies only to the mucin group, will be found in the future to be correct. It is the opinion of HAMMARSTEN that it is better to wait for further research in this direction before we drop the generally accepted nomenclature and the usual subdivisions of the proteins.

cleavage an amino-sugar, which has been shown to be glucosamine in all but a few exceptions.[1] In the chondroproteins, on the contrary, the protein is united to chondroitin-sulphuric acid.

1. Mucin Substances.

Compared with the simple proteins the mucin substances are poorer in nitrogen and as a rule also have considerably less carbon. The carbohydrate complex, whose nature has been shown by the investigations of Fr. Müller[2] and his pupils, occurs, as it seems, in the mucin substances as a polysaccharide related to chitosan, which on hydrolytic cleavage yields glucosamine (chitosamine), and, at least in most cases, acetic acid also. The mucin substances differ very markedly among themselves, hence we divide them into two groups, the mucins and the mucoids.

The *true mucins* are characterized by the fact that their natural solutions, or solutions prepared by the aid of a trace of alkali, are mucilaginous, ropy, and give a precipitate with acetic acid which is insoluble in excess of acid or soluble only with great difficulty. The *mucoids* do not show these physical properties, and have other solubilities and precipitation properties. As we have intermediate steps between different protein bodies, so also we have such between true mucins and mucoids, and a sharp line cannot be drawn between these two groups.

It is just as difficult at present to draw a sharp line between the proteins and the mucins or mucoids, since we have been able to split off carbohydrate complexes from several proteins, and as proteins have been isolated from white of egg which yield more or less glucosamine. The very variable amounts of glucosamine obtained under various conditions from the crystalline ovalbumin seem to indicate that we are dealing with a contamination with a glycoprotein. This question requires further study before we can designate these proteins as glycoproteins.

True mucins are secreted by the larger mucous glands, by certain mucous membranes, and by the skin of snails and other animals. True mucin also occurs in the navel-cord. Sometimes, as in snails and in the membrane of the frog-egg (Giacosa) and perch-eggs (Hammarsten[3]),

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[1] See Schulz and Ditthorn, Zeitschr. f. physiol. Chem., 29; A. v. Ekenstein and Blanksma, Chem. Centralbl., 1907, 2. When both carbohydrate groups are simultaneously combined in one body, then probably we are not dealing with a chemical individual, but rather with a mixture.

[2] See Fr. Müller, Zeitschr. f. Biologie, 42, which contains all the pertinent literature, and also L. Langstein, Die Bildung von Kohlenhydraten aus Eiweiss, Ergebnisse der Physiologie, Jahr. I, Abt. 1.

[3] Giacosa, Zeitschr. f. physiol. Chem., 7; Hammarsten, Pflüger's Archiv., 36, and Skand, Arch. f. Physiol., 17.

a mother-substance of mucin, a mucinogen, has been found which may be converted into mucin by alkalies. Mucoid substances are found in certain cysts, in the cornea, the crystalline lens, white of egg, and in certain ascitic fluids. The so-called tendon-mucin, which, according to the investigations of LEVENE and of CUTTER, and GIES,[1] contains chondroitin-sulphuric acid or a related substance, cannot be classified as a mucin, but must, like the chondromucoid and the osseomucoid, be classified as chondroprotein. As the mucin question has not been sufficiently studied, it is at the present time impossible to give any positive statements in regard to the occurrence of mucins and mucoids, especially as without doubt in many cases non-mucinous substances have been described as mucins.

True Mucins. Thus far we have been able to obtain only a few mucins in a pure and unchanged condition, because of the reagents used. The elementary analyses of these mucins have given the following results:

	C	H	N	S	
Mucin from mucous membrane (air-passages)	48.26	6.91	10.70	1.40	(FR. MÜLLER [2])
Mucin from submaxillary	48.84	6.80	12.32	0.84	(HAMMARSTEN [2])
Mucin from snail	50.32	6.84	13.65	1.75	(HAMMARSTEN [2])
Synovial mucin	51.05	6.53	13.01	1.34	(v. HOLST [3])

MÜLLER obtained 35 per cent glucosamine from mucous-membrane mucin and 23.5 per cent from the submaxillary mucin.

By the action of superheated steam on mucin a carbohydrate, animal gum (LANDWEHR), is split off. This has not been substantiated by other investigators, such as HAMMARSTEN, FOLIN and FR. MÜLLER.[4] Instead of a non-nitrogenous gum a nitrogenous carbohydrate derivative was always obtained.

On boiling mucin with dilute mineral acids, acid albuminate and bodies similar to proteoses are obtained, besides a reducing substance which is not free glucosamine (STEUDEL [5]). By the action of strong acids upon mucins or mucoids OTORI [6] obtained several of the cleavage products of the proteins, such as leucine, tyrosine, glycocoll, glutamic acid, oxalic acid, guanidine, arginine, lysine, and humus substances, and also carbohydrate cleavage products, such as levulinic acid. Certain mucins, as the submaxillary mucin, are easily changed by very dilute

[1] Levene, Zeitschr. f. physiol. Chem., **31**; Cutter and Gies, Amer. Journ. of Physiol., **6**.

[2] Fr. Müller, Zeitschr. Biologie, **42**; Hammarsten, Zeitschr. f. physiol. Chem., **12**, and Pflüger's Arch., **36**.

[3] Zeitschr. f. physiol. Chem., **43**.

[4] Landwehr, Zeitschr. f. physiol. Chem., 8, 9; also Pflüger's Arch., **39** and **40**; Folin, Zeitschr. f. physiol. Chem., **23**; Fr. Müller, Sitzungsber. d. Gesellsch. zur Beförd. d. gesammt. Naturwiss. zu Marburg, 1896.

[5] Zeitschr. f. physiol. Chem., **34**.

[6] *Ibid.*, **42** and **43**.

alkalies, as lime-water, while others, such as tendon-mucin, are not affected. If a strong caustic-alkali solution, such as 5-per cent KOH solution, is allowed to act on submaxillary mucin, we obtain alkali albuminate, bodies similar to proteoses and peptones and one or more substances of an acid reaction and with strong reducing powers.

On peptic digestion proteoses and peptone-like bodies, still containing the carbohydrate group, are produced. On tryptic digestion still simpler cleavage products are formed, namely, leucine, tyrosine, and tryptophane (Posner and Gies [1]). The glucosamine, so far as we know, is not split off by proteolytic enzymes, but only after strong hydrolysis with acids, and this speaks against the assumption that the glucosamine group exists as a glucoside-like combination in the mucin molecule (Neuberg and Milchner [2]).

In one or another respect the various mucins act somewhat dissimilarly. For example, the snail and sputum mucins are insoluble in dilute hydrochloric acid of 1–2 p. m., while the mucin of the submaxillary gland and the navel-cord is soluble. The former become flaky with acetic acid, while the submaxillary mucin is precipitated in more or less fibrous, tough masses. Still all the mucins have certain reactions in common.

In the dry state mucin forms a white or yellowish-gray powder. When moist it forms, on the contrary, flakes or yellowish-white tough lumps or masses. The mucins are acid in reaction. They give the color reactions of the proteins. They are not soluble in water, but may give a neutral solution with water with the aid of small amounts of alkali. Such a solution does not coagulate on boiling, but acetic acid gives at the normal temperature a precipitate which is nearly insoluble in an excess of the precipitant. If 5–10 per cent NaCl be added to a mucin solution, this can now be carefully acidified with acetic acid without giving a precipitate. Such acidified solutions are copiously precipitated by tannic acid; with potassium ferrocyanide they give no precipitate, but on sufficient concentration they become thick or viscous. A neutral solution of alkali mucin is precipitated by alcohol in the presence of neutral salts; it is also precipitated by several metallic salts. If mucin is heated on the water-bath with dilute hydrochloric acid of about 2-per cent, the liquid gradually becomes a yellowish or dark brown, and reduces copper salts in alkaline solutions.

The mucin most readily obtained in large quantities is the submaxillary mucin, which may be prepared in the following way: The filtered watery extract of the gland, free from form-elements and as colorless as possible, is treated with 25-per cent hydrochloric acid, so that the liquid contains 1.5 p. m. HCl. On the addition of the acid the mucin is immediately precipitated, but dissolves on stirring. If this acid liquid is imme-

[1] Amer. Journ. of Physiol., **11**.

[2] Berl. klin. Wochenschr., 1904.

diately diluted with 2–3 vols. of water, the mucin separates and may be purified by redissolving in 1–5 p. m. acid, and diluting with water and washing therewith. The mucin of the navel-cord may be prepared in the same way. As a rule the mucins can be prepared by precipitation with acetic acid and repeated solution in dilute lime-water or alkali, and reprecipitation with acetic acid. Finally they are treated with alcohol and ether. In the preparation of sputum mucin the method is very complicated (Fr. Müller).

The precipitation by acetic acid, as shown by Hammarsten,[1] is not applicable in the preparation of submaxillary mucin, because another protein substance is precipitated with the mucin, but remains in solution on using the hydrochloric-acid method above described. Posner and Gies [2] have by special experiments shown the power of mucins of precipitating proteins, and this makes the ordinary method of precipitating with acetic acid questionable.

Mucoids or **Mucinoids.** In this group we must include those non-phosphorized glycoproteins which are neither true mucins nor chondroproteids, although they show among themselves such differences in behavior that they can be divided into several subgroups of mucoids. To the mucoids belong *pseudomucin*, the probably related body *colloid*, *ovomucoid*, and other bodies, which on account of their differences will be best treated individually in their respective chapters.

Hyalogens. Under this name Krukenberg [3] has designated a number of different bodies, which are characterized by the following: By the action of alkalies they change, with the splitting off of sulphur and some nitrogen, into soluble nitrogenized products called by him *hyalines*, and which yield a pure carbohydrate by further decomposition. We find that very heterogeneous substances are included in this group. Certain of these hyalogens seem undoubtedly to be glycoproteins. *Neossin* [4] of the Chinese edible swallow's-nest, *membranin* [5] of Descemet's membrane and of the capsule of the crystalline lens, and *spirographin* [6] of the skeletal tissue of the worm Spirographis, seem to act as such. Others, on the contrary, such as *hyalin* [7] of the walls of hydatid cysts, and *onuphin*,[8] from the tubes of Onuphis tubicola, do not seem to be compound proteins. The so-called *mucin of the holothuriæ* [9] and *chondrosin* [10] of the sponge, Chondrosia reniformis, and others may also be classed with the hyalogens. As the various bodies designated by Krukenberg as hyalogens are very dissimilar, it is not of much advantage to arrange these in special groups.

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[1] Zeitschr. f. physiol. Chem., **12**.

[2] Amer. Journ. of Physiol., **11**.

[3] Verh. d. physik.-med. Gesellsch. zu Würzburg, 1883; also Zeitschr. f. Biologie, **22**.

[4] Krukenberg, Zeitschr. f. Biologie, **22**.

[5] C. Th. Mörner, Zeitschr. f. physiol. Chem., **18**.

[6] Krukenberg, Würzburg, Verhandl., 1883; also Zeitschr. f. Biologie, **22**.

[7] A. Lücke, Virchow's Arch., **19**; also Krukenberg, Vergleichende physiol. Stud., Series 1 and 2, 1881.

[8] Schmiedeberg, Mitth. aus d. zool. Stat. zu Neapel, **3**, 1882.

[9] Hilger, Pflüger's Archiv, **3**.

[10] Krukenberg, Zeitschr. f. Biologie, **22**.

2. Chondroproteins.

Chondroproteins are those glycoproteins which as primary cleavage products yield protein and an ethereal sulphuric acid containing a carbohydrate, *chondroitin-sulphuric acid. Chondromucoid,* occurring in cartilage, is the best example of this group. *Amyloid* occurring under pathological conditions also belongs to this group. On account of the property of chondroitin-sulphuric acid of precipitating protein, it is also possible that under certain circumstances combinations of this acid with protein may be precipitated from the urine and be considered as chondroproteins.

The chondromucoid, the so-called tendon-mucin, and the osseomucoid have greatest interest as constituents of cartilage, of the connective tissues, and the bones, and on this account these bodies and their cleavage product, chondroitin-sulphuric acid, will be treated in a following chapter (X). On the contrary, amyloid, which has always been considered in connection with the protein substances, will be described here.

Amyloid, so called by VIRCHOW, is a protein substance appearing under pathological conditions in the internal organs, such as the spleen, liver and kidneys, as infiltrations; and in serous membranes as granules with concentric layers. It probably also occurs as a constituent of certain prostate calculi. The chondroprotein occurring under physiological conditions in the walls of the arteries is, perhaps, according to KRAWKOW, very nearly related to the amyloid substance, but not identical with it, as shown by NEUBERG.[1]

Very recently O. HANSSEN[2] has studied the mechanically isolated amyloid obtained from the so-called " sago kernels " of an amyloid spleen, and could not detect any conjugated sulphuric acid in it. According to his investigations true amyloid is not a chondroprotein. On the other hand, he has found that amyloid organs (liver and spleen) are much richer in sulphuric acid that splits off than normal organs, and it is not improbable that the amyloid formation goes hand in hand with the formation of a chondroprotein. The question as to what relation this amyloid investigated by HANSSEN bears to such a chondroprotein and to the substances studied by others and called amyloid, requires further study, and the question as to the nature of the so-called amyloid is still unsettled. The amyloid protein prepared by MAYEDA[3] did not contain any chondroitin-sulphuric acid. It yielded histidine and did

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[1] Krawkow, Arch. f. exp. Path. u. Pharm., 40, which contains the literature; Neuberg, Verhandl. d. d. Pathol. Gesellsch., 1904.

[2] Biochem. Zeitschr., 13.

[3] Zeitschr. f. physiol. Chem., 58.

not, contrary to NEUBERG, behave like a histone. The quantity of hexone bases was not greater than in the proteins from normal organs. This amyloid protein yielded, like the amyloid degenerated tissues, some histone-peptone, and he claims that we have no foundation for the assumption that the amyloid protein has histone-like characteristics or is rich in bases. Under these circumstances it must be remarked that the following statements apply only to the results of older investigations.

The amyloid prepared by KRAWKOW and NEUBERG had about the same composition: C 49.0–50.1; H 7–7.2; N 14–14.1, and S 1.8–2.8 per cent. The aorta amyloid of man and of the horse contained respectively C 49.6 and 50.5; H 7.2; N 14.4 and 13.8; S 2.3 and 2.5 per cent. According to NEUBERG, aorta amyloid differs from spleen and liver amyloid by a different division of the nitrogen, which is evident from the following:

	Monamino-N.	Diamino-N.	Amide-N.
Liver amyloid.....	43.2	51.2	4.9
Spleen amyloid.....	30.6	57.0	11.2
Aorta amyloid.....	54.9	36.0	8.8

From liver amyloid NEUBERG obtained glycocoll 0.8; leucine 22.2; glutamic acid 3.8; tyrosine 4.0; proline 3.1; arginine 13.9, and lysine 11.6 per cent.

By the action of alkali, amyloid splits into protein and chondroitin-sulphuric acid (see Chapter X), and according to KRAWKOW it is therefore a firm, perhaps ester-like combination of this acid with protein. The protein, from the investigations of NEUBERG, is of a basic nature and most comparable to the histones. According to NEUBERG, amyloid is a transformation product of the proteins, just as are the protamines, and the differences between liver, spleen, and aorta amyloid indicate various phases of this transformation.

Amyloid is an amorphous white substance, insoluble in water, alcohol, ether, dilute hydrochloric and acetic acids. It is soluble in concentrated hydrochloric acid or caustic alkali with decomposition. On boiling with dilute hydrochloric acid it yields sulphuric acid and a reducing substance. It is not dissolved by gastric juice, according to KRAWKOW, which agrees with most of the older reports. It is nevertheless changed so that it is soluble in dilute ammonia, while the typical amyloid is insoluble therein. NEUBERG finds on the contrary that amyloid (from liver) is digested by pepsin as well as by trypsin, although more slowly than fibrin, and that it is also destroyed in autolysis, so that in life an absorption is possible. The amyloid from the "sago" spleen studied by HANSSEN showed the same behavior with gastric juice as KRAWKOW found, while trypsin, as well as autolysis for months, was without action.

Amyloid gives the xanthoproteic reaction and the reactions of MILLON and ADAMKIEWICZ. Its most important property is its behavior with certain coloring matters. It is colored reddish brown or a dingy violet by iodine; a violet or blue by iodine and sulphuric acid; red by methylaniline iodide, especially on the addition of acetic acid; and red also by aniline green. Of these color reactions those with aniline dyes are the most important. The iodine reaction appears less constant and is greatly dependent upon the physical condition of the amyloid. The color reactions are due to the presence of the chondroitin-sulphuric acid component, but this stands in opposition to the behavior of the amyloid obtained by HANSSEN from the " sago " spleen.

Amyloid may be prepared as follows, according to MODRZEJEWSKI and KRAWKOW.[1] The finely divided organ is exhausted first with water and then with dilute ammonia, which leaves the insoluble amyloid and removes the free or the combined chondroitin-sulphuric acid, besides other substances. The product, after being washed with water, is digested with pepsin for several days at 38° C. The residue, after washing with hydrochloric acid and water, is dissolved in dilute ammonia, filtered, again precipitated with dilute hydrochloric acid, dissolved, if necessary, in ammonia, precipitated a second time with hydrochloric acid, washed with water, the precipitate dissolved in baryta-water, which leaves the nucleins undissolved, and the barium filtrate precipitated with hydrochloric acid, and then washed with water, alcohol, and ether.

Phosphoglycoproteins. This group includes the phosphorized glycoproteins. They yield no purine bases (nuclein bases) as cleavage products. They are not nucleoproteins and therefore they must not be mistaken for them. On pepsin digestion they may, like certain nucleoalbumins, yield pseudonuclein, but they differ from the nucleoalbumins in that they yield a reducing substance on boiling with dilute acid. They differ from the nucleoproteins, which also yield reducing carbohydrates, in, as above stated, not yielding any purine bases.

Only two phosphorized glycoproteins are known at the present time, namely, *ichthulin*, occurring in carp eggs and studied by WALTER,[2] and which was considered as a vitellin for a time. Ichthulin has the following composition: C 53.52; H 7.71; N 15.64; S 0.41; P 0.43; Fe 0.10 per cent. In regard to solubilities it is similar to a globulin. WALTER has prepared a reducing substance from the pseudonuclein of ichthulin which gave a highly crystalline compound with phenylhydrazine.

Another phosphoglycoprotein is *helicoproteid*, obtained by HAMMARSTEN[3] from the glands of the snail Helix pomatia. It has the following composition: C 46.99; H 6.78; N 6.08; S 0.62; P 0.47 per cent. It is converted into a gummy, levorotatory carbohydrate, called *animal sinistrin*, by the action of alkalies. On boiling with an acid it yields a dextrorotatory reducing substance.

The compound protein found by SHULTZ and DITTHORN[4] in the spawn of the frog probably belongs to this group, but instead of glucosamine it gives galactosamine on cleavage.

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[1] Modrzejewski, Arch. f. exp. Path. u. Pharm., **1**; Krawkow, l. c.

[2] Zeitschr. f. physiol. Chem., **15**.

[3] Hammarsten, Pflüger's Arch., **36**.

[4] Zeitschr. f. physiol. Chem., **29**.

b. Nucleoproteins.

By this name we designate those compound proteins which yield protein and nucleic acid on cleavage. The nucleoproteins seem to be widely diffused in the animal body. They occur chiefly in the cell-nuclei, but they also often occur in the protoplasm. They may pass into the animal fluids on the destruction of the cells, hence nucleoproteins have also been found in blood serum and other fluids.

The nucleoproteins may be considered as combinations of a protein with a side-chain, which KOSSEL calls the *prosthetic group*. This side chain, which contains the phosphorus, may be split off as nucleic acid on treatment with alkali. The protein may be of different kinds. In certain cases this is histone, and the combinations between nucleic acid and protamines are also sometimes classified as nucleoproteins. The combination between protamine and nucleic acid is, it seems, a salt-like combination, and entirely different from the combination of the proteins with nucleic acid in the nucleoproteins. The following facts, given in connection with the nucleoproteins, do not apply to the nucleoprotamines. The nucleoproteins differ not only according to the protein component they contain, but also as to the nucleic acids, which vary among themselves. There are essentially different nucleic acids, some among which contain a pentose carbohydrate while others contain a hexose carbohydrate. The nucleic acids also differ in regard to the amount of purine and pyrimidine bases they contain (see below).

The native nucleoproteins contain a variable, but not a high percentage of phosphorus, which in most of the nucleoproteins investigated, ranges between 0.5 and 1.6 per cent. They also regularly contain iron, and in Octopodes HENZE[1] has observed an iron-free nucleoprotein with 0.96 per cent copper. The nucleoproteins behave like weak acids, especially those having considerable protein in the molecule. They therefore give the ordinary protein reactions and behave in this regard like the proteins. The nucleoproteins prepared from organs rich in cell nuclei seem to be characterized by containing more phosphorus and having a stronger acid character. All nucleoproteins are bodies that are insoluble in water, but whose alkali combination is soluble in water. From such a solution the nucleoprotein can be precipitated by acetic acid, and the precipitate dissolves with more or less difficulty and in some cases not at all, in an excess of the acid. It dissolves, on the contrary, in very dilute hydrochloric acid. In this respect nucleoproteins are similar to the nucleoalbumins and the mucin substances, but differ from these two groups in that they yield purine bases on hydrolysis. According to PLIMMER

[1] Zeitschr. f. physiol. Chem., 55.

and Scott [1] the nucleoproteins differ from the nucleo-albumins by the fact that with sodium hydroxide in 1-per cent solution the nucleoalbumins split off phosphoric acid while the nucleoproteins do not. The nucleoproteins give the color reactions of the proteins, but those which have been investigated are dextrorotatory and not levorotatory (Gamgee and Jones [2]).

The nucleoproteins are readily modified. The alkali combination soluble in water suffers a decomposition on heating its solution, when as neutral as possible, and coagulated protein separates and a protein rich in phosphorus and poor in protein with strong acid character remains in solution. By the action of weak acids and by gastric juice a similar cleavage takes place, whereby the protein split off goes into solution while the nucleoprotein rich in phosphorus, so-called *nuclein* (Miescher, Hoppe-Seyler [3]) or *true nuclein*, remains undissolved. As the nuclein is probably nothing but a partly modified nucleoprotein poorer in protein, having a composition varying with the intensity of the cleavage, it seems unnecessary to give the name nuclein thereto. On the other hand, the nucleins have other properties than the nucleoproteins, and as the nucleins bear the same relation to the neucleoproteins that the pseudonuclein does to the nucleoalbumins, we will give here a short description of the nucleins as well as the pseudo- or paranucleins.

Nucleins or true nucleins are formed, as above stated, from nucleoproteins in their peptic digestion or by treatment with dilute acids. It must be remarked that the nucleins are not entirely resistant toward gastric juice, and also that at least one nucleoprotein, namely, the one obtained from the pancreas, completely dissolves, leaving no nuclein residue on treatment with gastric juice (Umber, Milroy) [4]. The nucleins are rich in phosphorus, containing in the neighborhood of 5 per cent. According to Liebermann,[5] metaphosphoric acid can be split off from true nucleins (yeast nuclein). The nucleins are decomposed into protein and nucleic acid by caustic alkali, and as different nucleic acids exist, so also there exist different nucleins. As previously stated, proteins may be precipitated in acid solutions by nucleic acids, and in this way, as shown by Milroy, combinations of nucleic acid and proteins may be prepared which behave quite like true nucleins. All nucleins yield purine bases (so-called nuclein bases) on boiling with dilute acids. The nucleins contain iron to a considerable extent. They act like rather strong acids.

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[1] Plimmer and Scott, cited in Biochem. Centralbl., 8, p. 109.

[2] Hofmeister's Beiträge, 4.

[3] Hoppe-Seyler, Med. chem. Unters., 452.

[4] Umber, Zeitschr. f. klin. Med., 34; Milroy, Zeitschr. f. physiol. Chem., 22.

[5] Pflüger's Arch., 47.

The nucleins are colorless, amorphous and insoluble or only slightly soluble in water. They are insoluble in alcohol and ether. They are more or less readily dissolved by dilute alkalies. The nucleins give the biuret test and MILLON'S reaction. They show a great affinity for many dyes, especially the basic ones, and take these up with avidity from watery or alcoholic solutions. On burning they yield an acid residue which is very difficult to incinerate and which contains metaphosphoric acid. On fusion with saltpeter and soda the nucleins yield alkali phosphates.

To prepare nucleins from cells or tissues, first remove the chief mass of proteins by artificial digestion with pepsin-hydrochloric acid, lixiviate the residue with very dilute ammonia, filter, and precipitate with hydrochloric acid. The precipitate is further digested with gastric juice, washed and purified by alternately dissolving in very faintly alkaline water and reprecipitating with an acid, washing with water, and treating with alcohol-ether. A nuclein may be prepared more simply by the digestion of a nucleoprotein. In the detection of nucleins we make use of the above-described method, testing for phosphorus in the product after fusing with saltpeter and soda. Naturally the phosphates and phosphatides must first be removed by treatment with acid, alcohol, and ether, respectively. We must specially call attention to the fact, as shown by LIEBERMANN,[1] that it is very difficult to remove lecithin (the phosphatides) by means of alcohol-ether. No exact methods are known for the quantitative estimation of nucleins in organs or tissues.

Pseudonucleins or PARANUCLEINS. These bodies are obtained as an insoluble residue on the digestion of certain nucleoalbumins or phosphoglycoproteins with pepsin-hydrochloric acid. Attention is called to the fact that the pseudonuclein may be dissolved by the presence of too much acid or by a too energetic peptic digestion. If the relation between the degree of acidity and the quantity of substance is not properly selected, the formation of pseudonucleins may be entirely overlooked in the digestion of certain nucleoalbumins. Pseudonucleins contain phosphorus, which, as shown by LIEBERMANN,[2] is split off as metaphosphoric acid by mineral acids.

The pseudonucleins are amorphous bodies insoluble in water, alcohol, and ether, but readily soluble in dilute alkalies and barium hydroxide solution. They are readily split by barium hydroxide solution with the splitting off of phosphoric acid, and according to GIERTZ[3] they differ in this regard from the true nucleins, which are neither dissolved nor decomposed by baryta. They are not soluble in very dilute acids, and may be precipitated from their solution in dilute alkalies by adding acid. They give the protein reactions very strongly, but do not yield purine bases.

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[1] Pflüger's Arch., **47**.

[2] Ber. d. d. chem. Gesellsch., **21**, and Centralbl. f. d. med. Wissensch., 1889.

[3] Zeitschr. f. physiol. Chem., **28**.

In preparing a pseudonuclein, dissolve the mother-substance in hydrochloric acid of 1–2 p. m., filter if necessary, add pepsin solution, and allow the mixture to stand at the temperature of the body for about twenty-four hours. The precipitate is filtered off, washed with water, and purified by alternately dissolving in very faintly alkaline water and reprecipitating with acid.

Plastin. After the extraction of the nucleins from cell nuclei of certain plants by dilute soda solution, a residue is obtained which is characterized by its great insolubility. The substance which forms this residue has been called plastin. This substance, of which the spongioplasm of the body of the cell and the nucleus granules are alleged to be composed, is considered as a nuclein modification of great insolubility, although its nature is not known.

Cleavage Products of the Nucleoproteins.

1. The Nucleic Acids.

All nucleic acids are rich in phosphorus and yield phosphoric acid, purine bases and a carbohydrate or carbohydrate derivative, as cleavage products. Certain of them also contain pyrimidine bases. Not only do the nucleic acids differ among themselves in regard to the occurrence of different purine bases, but the opinions of authorities concerning this difference are conflicting. This last is perhaps due to the fact, which is now admitted, that the two purine bases xanthine and hypoxanthine can be produced secondarily from guanine and adenine. The statements as to the occurrence of more than two purine bases in a nucleic acid require further confirmation. There is no doubt that the most thoroughly studied nucleic acids, such as the thymus-nucleic acids, the closely related or perhaps identical acids of the salmon sperm (salmo-nucleic acid), of the herring sperm and burbot sperm, and of the pancreas, do not contain more than two purine bases, namely, guanine and adenine.

Guanylic acid and inosinic acid contain only one purine base, namely guanine and hypoxanthine respectively. The simplest nucleic acids also contain no pyrimidine bases, which otherwise are found thus far in all carefully investigated nucleic acids. Not all the nucleic acids are the same in respect to the pyrimidine bases they contain. Although thymine, cytosine and uracil are regularly obtained, and it is known that the uracil can be produced secondarily from the cytosine, still the plant nucleic acid, the triticonucleic acid, yields only cytosine and uracil, but no thymine according to Osborne and Heyl,[1] who are confirmed by Wheeler and Johnson.[2] Levene and Mandel[3] have also found cytosine and uracil, but no thymine, in the nucleic acid from the eggs of the haddock.

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[1] Amer. Journ. of Physiol., **21**.
[2] Amer. Chem. Journ., **29**.
[3] Zeitschr. f. physiol. Chem., **49**.

Pentoses have been found as the carbohydrate group in the guanylic acid, the inosinic acid, and the plant nucleic acids (yeast and triticonucleic acids), and a hexose has also been found in the yeast nucleic acid. In the other animal thymo-nucleic acids, a hexacarbohydrate occurs, as shown by the investigations of STEUDEL.[1] We can differentiate between two different groups of nucleic acids according to the kind of carbohydrate complexes they contain.

Another difference also exists, as above shown, in the number and kind of purine and pyrimidine bases in the nucleic acids. According to the number of these bases we can differentiate between the simple nucleic acids with only one base and complex nucleic acids with several bases. LEVENE and MANDEL[2] designate the first group *nucleotides* or *mononucleotides* and the second group *polynucleotides*. As we are in the habit of designating as *nucleotin* or *nucleotinic* acid (see below), only those cleavage products of nucleic acids containing no purine base, but merely pyrimidine bases, it is not proper, according to HAMMARSTEN, to designate as nucleotides such bodies as guanylic acid and inosinic acid, which contain no pyrimidine, but only purine base. Until the nature of the nucleic acids is completely explained, it would be better to differentiate between mono- and polynucleic acids, or perhaps still better between simple and complex nucleic acids. Yet we must bear in mind that the complex nucleic acids have not been isolated from compound proteins, but generally from organs, namely, from mixtures of several nucleoproteins; hence we cannot know whether these acids are chemical individuals or whether they are mixtures of closely related simple nucleic acids. On the other hand, it is also possible that the simple nucleic acids may be derived from more complex ones by cleavage. Such an assumption does not apply to the guanylic acid, as its mother protein contains only one base, namely guanine.

We generally admit of 4 atoms of phosphorus in the empirical formula of the various nucleic acids. The relationship of phosphorus to nitrogen in the thymus- and the salmo-nucleic acid is according to SCHMIEDEBERG 4 to 14, and according to STEUDEL 4 to 15. OSBORNE and HARRIS found the relationship 4 to 16 in the triticonucleic acid, and BANG[3] found the relationship 4 to 20 in the guanylic acid.

All nucleic acids are amorphous, white, and have an acid reaction. They are readily soluble in ammoniacal or alkaline water. They also dissolve in concentrated acetic acid and form insoluble salts with copper

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[1] Zeitschr. f. physiol. Chem., Bd., **50**, **52**, **53**, **55**, **56**.

[2] Ber. d. d. chem. Gesellsch., **41**.

[3] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **37**, **43**, **57**; Steudel, Zeitschr. f. physiol. Chem., **49**, **53**, p. 14; Orborne and Harris, *ibid.*, **36**; Bang, *ibid.*, **26** and **31**, and Hofmeister's Beiträge, **5** and Biochem. Centralbl., **1**, p. 295.

chloride and salts of the heavy metals, and as a rule insoluble basic salts with the alkaline earths. The β-guanylic acid is soluble with difficulty in cold water but rather readily in boiling water, from which it separates on cooling. Guanylic acid is readily precipitated from its alkali compound by an excess of acetic acid. The other nucleic acids are, on the contrary, not precipitated from such compounds by an excess of acetic acid, but by a slight excess of hydrochloric acid, especially in the presence of alcohol. In acid solutions these latter nucleic acids give precipitates with proteins, which are considered as nucleins. The behavior of guanylic acid in this regard has not been shown on account of the great difficulty in dissolving it in dilute acids. All nucleic acids are insoluble in alcohol and ether. They do not give either the biuret test or MILLON'S reaction. The nucleic acids are optically active and indeed dextrorotatory (GAMGEE and JONES [1]).

The proteolytic enzymes, such as pepsin and trypsin, decompose the nucleoproteins more or less; the nucleic acids are not split by these enzymes as far as phosphoric acid and purine bases. Such a cleavage can, on the contrary, be brought about by erepsin (NAKAYAMA) or by other closely allied enzymes which have been called *nucleases* (IWANOFF, FR. SACHS). Micro-organisms can also bring about a more or less deep cleavage of the nucleic acids (SCHITTENHELM and SCHRÖTER [2]).

The animal nucleic acids, with the exception of guanylic acid and inosinic acid, are very closely related to each other, although they are not identical. They all yield thymine as a cleavage product; and as the most studied representative are the nucleic acids of the thymus gland (thymus nucleic acids) it is advisable for the present to treat them as one group, which has received the common name of thymo-nucleic acids.

Thymonucleic Acids. The most important investigations on the nucleic acids have been carried out by KOSSEL and his collaborators, by MIESCHER, SCHMIEDEBERG, STEUDEL, LEVENE, and LEVENE and MANDEL.[3] A. NEUMANN has isolated two nucleic acids, α- and β-thymus

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[1] Proc. Roy. Soc., 72.

[2] Nakayama, Zeitschr. f. physiol. Chem., 41; Iwanoff, *ibid.*, 39; Fr. Sachs, "Ist die Nuklease mit dem Trypsin identisch?" Inaug.-Dissert, Heidelberg, 1905; Schittenhelm and Schröter, f. physiol. Zeitschr. Chem., 41.

[3] The work of Kossel and his pupils on the nucleic acids can be found in: Arch. f. (Anat. u.) Physiol, 1892, 1893; Sitz. Ber. d. Berl. Akad. d. Wiss., 18, 1894; Centralbl. f. d. med. Wiss. 1893; Ber. d. d. chem. Gesellsch., 26 and 27; Zeitschr. f. physiol. Chem., 22 and 38; see also Neumann, Arch. f. (Anat. u.) Physiol., 1898 and 1899 Suppl.; Miescher, Hoppe-Seyler's Med. chem. Unters., p. 441 and Arch. f. exp. Path. u. Pharm., 37; Schmeideberg, *ibid.*, 37, 43, and 57; Altman, Arch. f. (Anat. u.) Physiol., 1889; Ascoli, Zeitschr. f. physiol. Chem., 28 and 31; Levene, *ibid.*, 32, 37, 38, 39, 43, 45; Levene and Mandel, *ibid.*, 46, 47, 49, 50; Inouye and Kotake, *ibid.*, 46; Steudel, *ibid.*, 42, 43, 46, 49, 50, 52, 53, 55, 56.

nucleic acid, from the thymus gland. The α-acid is soluble with difficulty, and in proper concentration gives a sodium salt which gelatinizes, and a barium salt which is precipitated by barium acetate in substance (KOSTYTSCHEW). The barium salt of the β-acid is not precipitated by barium acetate. The α-acid is designated as anhydric by SCHMIEDEBERG[1], and the β-acid as hydrate, and the first can be transformed into the second by heating. This transformation, according to KOSTYTSCHEW, is a decomposition whereby two-thirds of the purine bases are split off.

According to SCHMIEDEBERG the thymus nucleic acid is identical with the salmo-nucleic acid (from salmon sperm), and also according to STEUDEL probably with the acid from the herring sperm. Other nucleic acids, at least very closely related to this nucleic acid, have been prepared from the sperm of the burbot (Lota vulgaris) by ALSBERG, of the sturgeon (NOLL) and of the sea-urchin (MATHEWS), also from ox-sperm, brain, spleen (LEVENE), pancreas (LEVENE, v. FÜRTH and JERUSALEM, STEUDEL), mammary glands and kidneys (LEVENE and MANDEL[2]) and from other organs.

We are at the present time not agreed as to the formula for the most carefully studied thymonucleic acids (from the thymus, herring and salmon sperm). According to the numerous analyses of SCHMIEDEBERG and his collaborators for every 4 atoms of phosphorus there occur 14 atoms of nitrogen. The relationship of C to P was 40 to 4 and the relation C to N in 12 out of 15 preparations was 40 to 14, and only in 3 preparations 40 to 15. From these facts SCHMIEDEBERG has given the acid the formula $C_{40}H_{56}N_{14}O_{16}.2P_2O_5$. According to STEUDEL for every 4 atoms of phosphorus we have 15 atoms nitrogen and from this he has calculated the formula $C_{43}H_{57}N_{15}O_{12}.2P_2O_5$ for the thymonucleic acids. The results obtained on elementary analysis are not sufficient to decide between these two formulæ.

On the decomposition of the nucleic acids the purine bases are first more or less completely split off and correspondingly also various intermediary products are obtained. One of these is the *heminucleic acid* obtained by ALSBERG and containing only one-half of the purine bases, and another body is *thymic acid*, which is obtained on heating the free acid with water, when guanine, adenine and cytosine are simultaneously split off. Thymic acid forms a barium salt soluble in water, having the formula $C_{16}H_{23}N_3P_2O_{12}.Ba$ (KOSSEL and NEUMANN[3]).

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[1] Neumann, Arch. f. (Anat. u.) Physiol., 1898 and 1899 Suppl.; Kostytschew, Zeitschr. f. physiol. Chem., **39**; Schmiedeberg, l. c.

[2] Alsberg, Arch. f. exp. Path. u. Pharm., **51**; Noll, Zeitschr. f. phyisol. Chem., **25**; Mathews, *ibid.*, **23**; v. Fürth and Jerusalem, Hofmeister's Beiträge, **10** and **11**; Steudel, Zeitschr. f. physiol. Chem., **53**. See also foot-note 3, p. 176.

[3] Alsberg, l. c.; Kossel and Neumann, Zeitschr. f. physiol. Chem., **22**.

On more energetic cleavage, ALSBERG was able to isolate a phosphorus-free product, *nucleotin*, $C_{30}H_{42}N_4O_{13}$, which contained no purine bases and which, according to SCHMIEDEBERG, is the ground substance of the nucleic acid. On the other hand, as shown by STEUDEL, products may be obtained by the action of nitric acid which contain nearly all of the phosphoric acid in organic combination with the carbohydrate complexes. Such a cleavage product containing phosphoric acid and carbohydrate is of special interest, as it also contained thymine, has been obtained by LEVENE and MANDEL on the acid cleavage of thymus nucleic acid, and called *glycophosphothymic acid*. The relation of this substance to the thymic acid has not been investigated.

KUTSCHER and SEEMANN obtained guanidine and urea, but no uric acid, as products on the oxidation of nucleic acid with potassium permanganate. KUTSCHER and SCHENCK [1] besides these also obtained adenine, oxalic acid, acetic acid, an acid having an unknown formula, and another acid which they call *martamic acid*, besides guanidine and urea. *Martamic acid* has the formula $C_5H_8N_6O_5$ or $C_4H_{10}N_6O_5$ and gives a silver salt which is soluble in ammonia or nitric acid, and which crystallizes in tufts of leaves. The crystalline acid, which is soluble in ether, sublimes at 150°, and does not give the murexide test or Weidel's test.

Nothing decisive is known as to the constitution of the thymonucleic acids. According to SCHMIEDEBERG they are combinations of phosphoric acid with the above-mentioned nucleotin, a nucleotin-phosphoric acid, with which the purine bases are combined in some way or another.

STEUDEL on the contrary considers them as a tetrametaphosphoric acid, each phosphorus atom having a carbohydrate group (a hexa carbohydrate) and also one of the four nitrogenous cleavage products—guanine, adenine, cytosine and thymine—attached. This view is undoubtedly very promising, and if the statement of LEVENE and MANDEL on the formation of a glycophosphothymic acid as a cleavage product of the thymus nucleic acid is substantiated by further research, then this view of STEUDEL will receive further support. LEVENE and MANDEL admit the existence of other such mononucleotides, namely, of glycophospho-adeninic, -cytosinic, and -guaninic acids, which are combined with each other, forming a complex nucleic acid, a polynucleotide. This view is very similar to that held by STEUDEL. The question whether the complex, sometimes called true nucleic acids, are chemical individuals or are only mixtures of related, simpler nucleic acids has become of interest by these investigations of LEVENE and STEUDEL.

Guanylic Acid. This acid, which was first prepared by BANG from the pancreas, has, according to him, the composition $C_{44}H_{66}N_{20}P_4O_{34}$. It has also been found by JONES and ROWNTREE in the spleen and by

[1] Kutscher and Schenck, Zeitschr. f. physiol. Chem., **44**; Kutscher and Seemann, Ber. d. d. chem. Gesellsch., **36**, and Centralbl. f. Physiol., **17**.

LEVENE and MANDEL[1] in the liver. As positive cleavage products it yields guanine, pentose (*l*-xylose according to NEUBERG) and phosphoric acid. According to LEVENE and JACOBS[2] guanylic acid consists of a combination of phosphoric acid and the nucleoside, *guanosin*, $C_{10}H_{13}N_2O_5$, which is a glucoside-like combination of guanine and pentose (*d*-ribose). As to the occurrence of glycerin among the cleavage products nothing positive is known, and in fact certain investigations disprove the occurrence of this substance.[3] The acid first described by BANG, the β-acid, is formed from another acid, the α-guanylic acid, with a splitting off of 1 molecule pentose, and the α-guanylic acid contains 1 molecule pentose and 1 molecule guanine for every molecule of phosphoric acid. Correspondingly, as pentose is split off in its formation, the β-acid is somewhat richer in phosphorus and nitrogen than the α-acid, namely, 7.64 per cent P and 18.21 per cent N as compared with 6.65 per cent P and 15.38 per cent N in the α-acid. This latter acid differs from the β-acid in being readily soluble in cold water.

According to BANG[4] the thymus also contains a simple nucleic acid, namely, an adenylic acid; still this is not based on complete investigations.

Inosinic Acid, $C_{10}H_{13}N_4PO_8$ was first isolated by LIEBIG from the flesh of certain animals and then closely studied by HAISER.[5] It is obtained from beef extracts, and according to the investigations of NEUBERG and BRAHN, FR. BAUER, and LEVENE and JACOBS it is a simple nucleic acid.[6] On hydrolysis it yields phosphoric acid, hypoxanthine and pentose, according to the equation:

$$C_{10}H_{13}N_4PO_8 + 2H_2O = H_3PO_4 + C_5H_4N_4O + C_5H_{10}O_5.$$

The pentose is according to NEUBERG and BRAHN *l*-xylose, but LEVENE and JACOBS[7] have conclusively shown that the pentose is not *l*-xylose, but that it is *d*-ribose.

Inosinic acid is amorphous, and its solution, containing hydrochloric acid is levogyrate: $(\alpha)_D = -18.5°$. It gives crystalline salts, among

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[1] Bang, Zeitschr. f. physiol. Chem., **26**; with Raaschou, Hofmeister's Beiträge, **4**; Jones and Rowntree, Journ. of biol. chem., **4**; Levene and Mandel, Biochem. Zeitschr. **10**.

[2] Ber. d. d. chem. Gesellsch., **42**, 2469.

[3] v. Fürth and Jerusalem, Hofmeister's Beiträge, **10** and **11**; Steudel, Zeitschr. f. physiol. Chem., **53**. See also Bang, Hofmeister's Beiträge, **11**.

[4] Hofmeister's Beiträge, **5**.

[5] Liebig, Annal. d. chem. u. Pharm., **62**; Haiser, Monatsh. f. chem., **16**.

[6] Neuberg and Brahn, Biochem. Zeitschr., **5** and Ber. d. d. chem. Gesellsch., **41**, p. 3376; Bauer, Hofmeister's Beiträge, **10**; Levene and Jacobs, Ber. d. d. chem. Gesellsch., **41**, p. 2703.

[7] Levene and Jacobs, Ber. d. d. chem. Gesellsch., **42**.

which the barium salt, which is difficultly soluble in water, must be mentioned.

According to NEUMANN, the two thymus nucleic acids, α and β, can be obtained from the gland, after previously boiling the latter with water containing acetic acid and then cutting it up fine. The finely divided gland is boiled with about 3-per cent NaOH for one-half hour for the α-acid and two hours for the β-acid, and sodium acetate is added at the same time. After neutralization with acetic acid, filtration and concentration, the product is precipitated with alcohol. The nucleic acids can be obtained from the precipitated sodium nucleates by precipitating with alcohol containing hydrochloric acid. In the separation of the two acids, KOSTYTSCHEW makes use of the different behavior of the barium salts on saturating their solution with barium acetate (see above). LEVENE's method consists, on the contrary, in treating the organs first with 5-per cent sodium hydroxide or with 8-per cent ammonia in the cold, then nearly neutralizing with acetic acid, precipitating the proteins with picric acid, and treating the strongly acidified liquid (acetic acid) with alcohol. In the presence of sufficient acetate the nucleic acids are precipitated. More recently LEVENE has suggested that the nucleic acid be dissolved in strong acetic acid and then precipitated with copper chloride or hydrochloric acid. SCHMIEDEBERG (in collaboration with HERLANT) who has suggested a careful method for the preparation of the nucleic acids as copper compounds, has recently [1] given very exact and detailed instruction as to the preparation of the nucleic acids. In regard to the details for the various methods of preparation we must refer to the original publications cited below.

Guanylic acid may be best prepared, according to BANG and RAASCHOU,[2] by the following method: After treating the pancreas with 1-per cent sodium-hydroxide solution for twenty-four hours at the room temperature, it is dissolved by warming, then made faintly acid with acetic acid, filtered, made faintly alkaline with ammonia, strongly concentrated, and precipitated with alcohol while hot. The proteoses remain in solution, and the precipitated guanylic acid (α-acid) is purified by repeated solutions in water and precipitations by alcohol.

In regard to the preparation of inosinic acid we refer to the works of HAISER, of NEUBERG and BRAHN and of LEVENE and JACOBS cited on page 179.

Plant Nucleic Acids. Those best known are the yeast nucleic acid and the triticonucleic acid, $C_{41}H_{61}N_{16}P_4O_{31}$, isolated by OSBORNE and HARRIS from the wheat embryo, and which according to these investigators is identical with the yeast nucleic acid. Yeast nucleic acid has the probable formula $C_{38}H_{50}N_{15}P_4O_{29}$, according to LEVENE.[3] Instead of *l*-xylose it contains *d*-ribose as the pentose,

[1] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **43** and **57**; Herlant, *ibid.*, **44**; Neumann, Arch. f. (Anat. u.) Physiol., 1899, Suppl.; Levene, Zeitschr. f. physiol.Chem., **32** and **45**; Kostytschew, *ibid.*, **39**.

[2] Hofmeister's Beiträge, **4**.

[3] Biochem. Zeitschr., **17**.

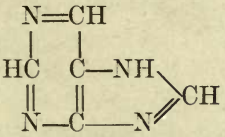
and LEVENE and JACOBS [1] have prepared two nucleosides from this acid, *guanosin* and *adenosin*, which have an analogous structure to *inosin*, namely, consist of pentose combined with the corresponding purine base, as indicated by the name. All three nucleosides, inosin, guanosin and adenosin are levorotatory. The plant nucleic acids are closely related to the thymonucleic acids, but differ from them by the fact that in the thymonucleic acids the pyrimidine groups are represented by uracil, cytosine, and thymine, and in the triticonucleic acid by cytosine and uracil. This last acid, which is dextrorotatory, yields on hydrolysis guanine, adenine, cytosine and uracil. (WHEELER and JOHNSON, OSBORNE and HEYL.[2]) As this last can be formed from the cytosine it is possible that only the first three bases exist preformed in the acid. An acid with adenine, guanine and two molecules cytosine also corresponds to the formula where the relationship of P to N is 4 to 16. LEVENE has been able to prepare from the tubercle bacilli nucleic acids whose nature has not been closely studied.

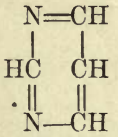
Plasminic acid is an acid which was prepared by ASCOLI and KOSSEL [3] by the action of alkali upon yeast. It contains iron, and is soluble in very dilute hydrochloric acid (1 p. m.). It is still a question whether it is a mixture or a chemical individual.

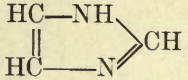
In regard to the preparation of yeast and triticonucleic acid we must refer to the works of ALTMANN, KOSSEL, OSBORNE and HARRIS. [4]

2. Purine Bases.

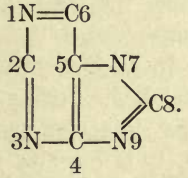
The cleavage products obtained from the nucleic acids, the *nuclein bases*, which are also called *alloxuric bases* by KOSSEL and KRÜGER, are members of the larger group of *purines*, to which also belongs the uric acid which is a substance occurring in the animal body. The constitution of these bodies has been explained by E. FISCHER, [5] and he has prepared many of the bodies synthetically. They can all be derived from the synthetically prepared purine, $C_5H_4N_4$, which has the formula given below and which may be considered as a combination of a pyrimidine ring with an imidazole ring.


Purine

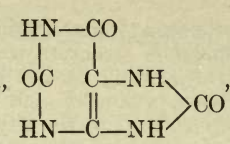
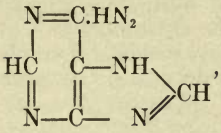
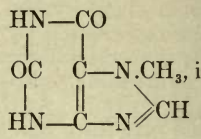

Pyrimidine


Imidazole

The different purine bodies are derived therefrom by the substitution of the various hydrogen atoms by hydroxyl, amide, or alkyl groups. In order to signify the different positions of substitution FISCHER has proposed to number the nine members of the purine nucleus in the following way:



[1] Ber. d. d. chem. Gesellsch., 42, 2477 and 2703.
[2] See foot-note 1, page 174.
[3] Ascoli, Zeitschr. f. physiol., Chem. 28.
[4] See foot-note 3, p. 176.
[5] See E. Fischer, Untersuchungen in der Puringruppe (1882–1906) Berlin, 1907.

For example, uric acid,  , is 2, 6, 8-trioxypurine, adenine,  , is 6-aminopurine, and heteroxanthine,  , is 7-methyl-2, 6-dioxypurine, etc.

The starting-point used by FISCHER for the synthetical preparation of the purine bases was 2, 6, 8-trichlorpurine, which is obtained, with 8-oxy-2, 6-dichlorpurine as an intermediary product, from potassium urate and phosphorus oxychloride.

The purine bodies or alloxuric bodies found in the animal body or its excreta are as follows: *Uric acid, xanthine, heteroxanthine, 1-methylxanthine, paraxanthine, guanine, epiguanine, hypoxanthine, episarkine, adenine* (and *carnine?*). The bodies *theobromine, theophylline,* and *caffeine,* occurring in the vegetable kingdom, stand in close relation to this group.

The composition of the purine bodies most important from a physiological standpoint is as follows:

Uric acid,	$C_5H_4N_4O_3$	2, 6, 8-trioxypurine
Xanthine,	$C_5H_4N_4O_2$	2, 6-dioxypurine
1-methylxanthine,	$C_6H_6N_4O$	1-methyl " "
Heteroxanthine,	$C_6H_6N_4O_2$	7 " " "
Theophylline,	$C_7H_8N_4O_2$	1, 3-dimethyl " "
Paraxanthine,	$C_7H_8N_4O_2$	1, 7- " " "
Theobromine,	$C_7H_8N_4O_2$	3, 7- " " "
Caffeine,	$C_8H_{10}N_4O_2$	1, 3, 7-trimethyl " "
Hypoxanthine,	$C_5H_4N_4O$	6-oxypurine
Guanine,	$C_5H_5N_5O$	2-amino " "
Epiguanine,	$C_6H_7N_5O$	7-methyl " " " "
Adenine,	$C_5H_5N_5$	6-aminopurine
Episarkine,	$C_4H_5N_4O_3$(?)	
Carnine,	$C_7H_8N_4O_3$	

After SALOMON[1] had shown the occurrence of xanthine bodies in young cells, the importance of the purine bases as decomposition products of cell nuclei and of nucleins was shown by the pioneering researches of KOSSEL, who discovered adenine and theophylline. In those tissues in which, as in the glands, the cells have kept their original state, the purine bases are not found free, but in combination with other atomic groups (nucleic acids). In such tissues, on the contrary, as in muscles, which are poor in cell nuclei, the purine bases are found in the free state. Since the purine bases, as suggested by KOSSEL, stand in close relationship to the cell nucleus, it is easy to understand why the quantity of these bodies is so greatly increased when large quantities of nucleated

[1] Sitzungsber. d. Bot. Verein der Provinz Brandenburg, 1880.

cells appear in such places as were before relatively poorly endowed. As an example of this, the blood, in leucæmia, is extremely rich in leucocytes. In such blood KOSSEL [1] found 1.04 p. m. purine bases, against only traces in the normal blood. That these bases are also intermediate steps in the formation of uric acid in the animal organism is probable, and will be shown later (see Chapter XV).

Only a few of the purine bases have been found in the urine or in the muscles. Only four bases—xanthine, guanine, hypoxanthine, and adenine—have been obtained, thus far, as cleavage products of nucleins, and these do not always have a primary origin. In regard to the purine bodies from other substances we refer the reader to their respective chapters. Only the above four bodies, the real nuclein bases, will be considered at this time.

Of these four bodies xanthine and guanine form one special group and hypoxanthine and adenine another. By the action of nitrous acid guanine is converted into xanthine and adenine into hypoxanthine.

$$\underset{\text{Guanine}}{C_5H_4N_4O.NH} + HNO_2 = \underset{\text{Xanthine}}{C_5H_4N_4O_2} + N_2 + H_2O;$$

$$\underset{\text{Adenine}}{C_5H_4N_4.NH} + HNO_2 + \underset{\text{Hypoxanthine}}{C_5H_4N_4O} + N_2 + H_2O.$$

Similar transformation, where xanthine and hypoxanthine are produced secondarily, may also occur in the hydrolysis of nucleic acids as well as in putrefaction and by the action of special enzymes. The researches of SCHITTENHELM, LEVENE, JONES, PARTRIDGE, WINTERNITZ, and BURIAN [2] have shown that in various organs deamination enzymes, such as *guanase* and *adenase*, occur, which convert guanine and adenine into xanthine and hypoxanthine respectively, and also *oxidases* which oxidize hypoxanthine into xanthine and this then into uric acid. This formation of uric acid from the purine bases, which will be discussed in detail in a following chapter (XV), is of very great interest. In this connection we must also call attention to the fact, as shown by SUNDVIK,[3] that by reduction of uric acid in alkaline solution two bodies may be obtained which, although not quite identical with xanthine and hypoxanthine, are at least bodies very similar thereto.

According to BURIAN [4] the purine bases give beautiful red products with diazo-compounds as long as the imide hydrogen in the 7th position (see structural formula above) is not substituted. As the nucleic acids do not react with the diazo compounds, BURIAN concludes that prob-

[1] Zeitschr. f. physiol. Chem., **7**.

[2] See Chapter XV (uric acid formation).

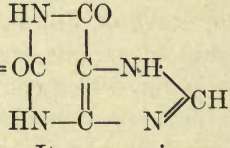
[3] Zeitschr. f. physiol. Chem., **23**.

[4] Ber. d. d. chem. Gesellsch., **37** and Zeitschr. f. physiol. Chem., **42** and **51**.

ably the nucleic-acid residue is combined with the imide hydrogen at position 7.[1]

HANS FISCHER[2] has found that the combinations of the purine bases with diazobodies are azo pigments, and that the diazo group enters at the 8th position. The purine bases substituted at the 8th position do not combine with diazo bodies any more than those substituted at the 7th position, and FISCHER concludes from this, contrary to BURIAN, that the nucleic acid molecule, which also does not react with diazo bodies, with the formation of pigments, can have the purine bases combined in the 8th as well as the 7th position.

The nuclein bases form crystalline salts with mineral acids, which, with the exception of the adenine salts, are decomposed by water. They are easily dissolved by alkalies, while with ammonia their action is somewhat different. They are all precipitated from acid solution by phosphotungstic acid; they also separate as silver compounds on addition of ammonia and ammoniacal silver-nitrate solution. These precipitates are soluble in boiling nitric acid of 1.1 specific gravity. All purine bodies are also precipitated by FEHLING'S solution (see Chapter XV) in the presence of a reducing substance such as hydroxylamine (DRECHSEL and BALKE). Copper sulphate and sodium bisulphite may also be used to advantage in their precipitation (KRÜGER).[3] This behavior of the purine bases serves just as well as the behavior with the silver solution for their precipitation and preparation.

Xanthine, $C_5H_4N_4O_2$,  (2, 6-dioxypurine),[4] is found

in several cellular organs. It occurs in small quantities as a physiological constituent of urine, and it occasionally has been found as a urinary sediment, or calculus. It was first observed in such a stone by MARCET. Xanthine is found in larger amounts in a few varieties of guano (Jarvis guano).

Xanthine is amorphous, or forms granular masses of crystals, or may also, according to HORBACZEWSKI,[5] separate as masses of shining, thin, large rhombic plates with 1 mol. water of crystallization. It is very

[1] In regard to the disputed views see Steudel, Zeitschr. f. physiol. Chem., 48; Burian, *ibid.*, 42 and 51.

[2] Zeitschr. f. physiol. Chem., 60.

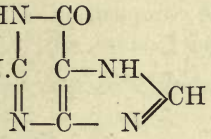
[3] Balke, Zur Kenntnis der Xanthinkörper, Inaug.-Diss. Leipzig, 1893; Krüger, Zeitschr. f. physiol. Chem., 18.

[4] In regard to the synthesis of xanthine and other purines see E. Fischer, foot-note 5, page 181.

[5] Zeitschr. f. physiol. Chem., 23.

slightly soluble in water, in 14,151–14,600 parts at 16° C., and in 1300–1500 parts at 100° C. (ALMÉN[1]). It is insoluble in alcohol or ether, but is readily dissolved by alkalies and with difficulty by dilute acids. With hydrochloric acid it gives a crystalline, difficultly soluble combination. With very little caustic soda it gives a readily crystallizable compound, which is easily dissolved by an excess of alkali. Xanthine dissolved in ammonia gives with silver nitrate an insoluble, gelatinous precipitate of silver xanthine. This precipitate is dissolved by hot nitric acid, and by this means an easily soluble crystalline double compound is formed. Xanthine in aqueous solution is precipitated on boiling with copper acetate. At ordinary temperatures xanthine is precipitated by mercuric chloride and by ammoniacal basic lead acetate. It is not precipitated by basic lead acetate alone.

When evaporated to dryness in a porcelain dish with nitric acid, xanthine gives a yellow residue, which turns, on the addition of caustic soda, first red, and, after heating, purple-red. If we place some chlorinated lime with some caustic soda in a porcelain dish and add the xanthine to this mixture, at first a dark-green and then quickly a brownish halo forms around the xanthine grains and finally disappears (HOPPE-SEYLER). If xanthine is warmed in a small vessel on the water-bath with chlorine-water and a trace of nitric acid, and evaporated to dryness, and the residue is then exposed under a bell-jar to the vapors of ammonia, a red or purple-violet color is produced (WEIDEL'S reaction). E. FISCHER[2] has modified WEIDEL'S reaction in the following way: He boils the xanthine in a test-tube with chlorine-water or with hydrochloric acid and a little potassium chlorate, then evaporates the liquid carefully, and moistens the dry residue with ammonia.

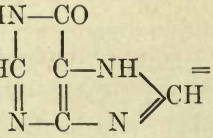
Guanine, $C_5H_5N_5O$, =  (2-amino-6-oxypurine).

Guanine is found in organs rich in cells. It is further found in the muscles (in very small amounts), in the scales and in the air-bladder of certain fishes, as iridescent crystals of guanine-lime; in the retinal epithelium of fishes, in guano, and in the excrement of spiders it is found as chief constituent. It also occurs in human and pig urine. Under pathological conditions it has been found in leucæmic blood, and in the muscles, ligaments, and articulations of pigs with guanine-gout.

Guanine is a colorless, ordinarily amorphous powder, which may be obtained as small crystals by allowing its solution in concentrated ammonia to evaporate spontaneously. According to HORBACZEWSKI it may under

[1] Journ. f. prakt. Chem., 96. [2] Ber. d. deutsch. chem. Gesellsch., 30, 2236.

certain conditions appear in crystals similar to creatinine-zinc chloride. It is insoluble in water, alcohol, and ether. It is rather easily dissolved by mineral acids and readily by alkalies, but it dissolves with great difficulty in ammonia. According to WULFF[1] 100 cc. of cold ammonia solution containing 1, 3, or 5 per cent NH_3 dissolve 9, 15, or 19 milligrams of guanine respectively. The solubility is relatively increased in hot ammonia solution. The hydrochloride readily crystallizes, and has been recommended by KOSSEL[2] for the microscopical detection of guanine, on account of its behavior toward polarized light. The sulphate contains 2 molecules of water of crystallization, which is completely expelled on heating to 120° C., and this fact, as well as the fact that guanine yields guanidine on decomposition with chlorine-water, differentiates it from 6-amino-2-oxypurine, which is considered as an oxidation product of adenine and possibly occurs as a chemical metabolic product (E. FISCHER). The 6-amino-2-oxypurine sulphate contains only 1 molecule of water of crystallization, which is not expelled at 120° C. Very dilute guanine solutions are precipitated by both picric acid and metaphosphoric acid. These precipitates may be used in the quantitative estimation of guanine. The silver compound dissolves with difficulty in boiling nitric acid, and on cooling the double compound crystallizes out readily. Guanine acts like xanthine in the nitric-acid test, but gives with alkalies on heating a more bluish-violet color. A warm solution of guanine hydrochloride gives with a cold saturated solution of picric acid a yellow precipitate consisting of silky needles (CAPRANICA). With a concentrated solution of potassium bichromate a guanine solution gives a crystalline, orange-red precipitate, and with a concentrated solution of potassium ferricyanide a yellowish-brown, crystalline precipitate (CAPRANICA). The composition of these and other guanine compounds has been studied by KOSSEL and WULFF.[3] It also gives a compound with picrolonic acid (LEVENE[4]). Guanine does not give WEIDEL'S reaction.

Hypoxanthine, SARKINE, $C_5H_4N_4O$, =  = (6-oxypurine).

This body has been found in all cellular organs and as a cleavage product of inosinic acid. It is especially abundant in the sperm of the salmon

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[1] Zeitschr. f. physiol. Chem., 17.

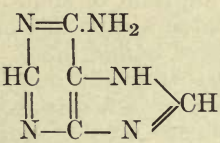
[2] Ueber die chem. Zusammensetz. der Zelle, Verh. d. physiol. Gesellsch. zu Berlin, 1890–91, Nos. 5 and 6.

[3] Zeitschr. f. physiol. Chem., 17; Capranica, *ibid.*, 4.

[4] Biochem. Zeitschr., 4.

and carp. Hypoxanthine occurs also in the marrow and in very small quantities in normal urine, and, as it seems, also in milk. It is found in rather considerable quantities in the blood and urine in leucæmia.

Hypoxanthine forms very small, colorless, crystalline needles. It dissolves with difficulty in cold water, but the claims concerning solubility therein are very contradictory.[1] It dissolves more readily in boiling water, in about 70–80 parts. It is nearly insoluble in alcohol, but is dissolved by acids and alkalies. The compound with hydrochloric acid is crystalline, and is more soluble than the corresponding xanthine derivative. It is easily soluble in dilute alkalies and ammonia. The silver compound dissolves with difficulty in boiling nitric acid. On cooling, a mixture of two hypoxanthine silver-nitrate compounds possessing an inconstant composition separates out. On treating this mixture with ammonia and an excess of silver-nitrate and heating, a silver hypoxanthine is formed, which when dried at 120° C. has a constant composition, $2(C_5H_2Ag_2N_4O)H_2O$, and is used in the quantitative estimation of hypoxanthine. Hypoxanthine picrate is soluble with difficulty, but if a boiling-hot solution of it is treated with a neutral or only faintly acid solution of silver nitrate the hypoxanthine is nearly quantitatively precipitated as the compound $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. Hypoxanthine does not yield an insoluble compound with metaphosphoric acid. When treated, like xanthine, with nitric acid, it yields a nearly colorless residue which, on warming with alkali, does not turn red. Hypoxanthine does not give WEIDEL's reaction. After the action of hydrochloric acid and zinc a hypoxanthine solution becomes first ruby-red and then brownish red in color on the addition of an excess of alkali (KOSSEL). According to E. FISCHER[2] a red coloration occurs even in the acid solution.

Adenine, $C_5H_5N_5$, 

(6-aminopurine), was first found by KOSSEL[3] in the pancreas. It occurs in all nucleated cells, but in greatest quantities in the sperm of the carp and in the thymus. Adenine has also been found in lucæmic urine (STADTHAGEN[4]). It may be obtained in large quantities from tea-leaves.

Adenine crystallizes with 3 molecules of water of crystallization in long needles which gradually become opaque in the air, but much more

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[1] See E. Fischer, Ber. d. deutsch. chem. Gesellsch., **30**.

[2] Kossel, Zeitschr. f. physiol. Chem., **12**, 252; E. Fischer, l. c.

[3] See Zeitschr. f. physiol. Chem., **10** and **12**.

[4] Virchow's Arch., **109**.

rapidly when warmed. If the crystals are warmed slowly with a quantity of water insufficient for solution, they suddenly become cloudy at 53° C., a characteristic reaction for adenine. It dissolves in 1086 parts cold water, but is easily soluble in warm. It is insoluble in ether, but somewhat soluble in hot alcohol and easily so in acids and alkalies. It is more easily soluble in ammonia solution than guanine, but less soluble than hypoxanthine. The silver compound of adenine is difficultly soluble in warm nitric acid, and deposits on cooling as a crystalline mixture of adenine silver-nitrates. With picric acid adenine forms a compound, $C_5H_5N_5.C_6H_2(NO_2)_3OH$, which is very insoluble and which separates more readily than the hypoxanthine picrate, and which can be used in the quantitative estimation of adenine. We also have an adenine mercury-picrate. Metaphosphoric acid with adenine gives a precipitate which dissolves in an excess of the acid if the solution is not too dilute. Adenine hydrochloride gives with gold chloride a double compound which consists in part of leaf-shaped aggregations and in part of cubical or prismatic crystals, often with rounded corners. This compound is used in the microscopic detection of adenine. With the nitric-acid test and with WEIDEL'S reaction adenine acts in the same way as hypoxanthine. The same is true for its behavior with hydrochloric acid and zinc with subsequent addition of alkali.

The procedure for the preparation and detection of the four above-described purine bases is, according to KOSSEL and his pupils, as follows: The finely divided organ or tissue is boiled for three or four hours with sulphuric acid of about 5 p. m. The filtered liquid is freed from protein by basic lead acetate, and the new filtrate is treated with sulphuretted hydrogen to remove the lead, again filtered, concentrated, and, after adding an excess of ammonia, precipitated with ammoniacal silver nitrate. The silver compound (with the addition of some urea to prevent nitrification) is dissolved in not too large a quantity of boiling nitric acid of sp. gr. 1.1, and this solution filtered boiling hot. On cooling, the silver xanthine remains in the solution, while the double compounds of guanine, hypoxanthine, and adenine crystallize out. The silver xanthine may be precipitated from the filtrate by the addition of ammonia and the xanthine set free by means of sulphuretted hydrogen. The three above-mentioned silver-nitrate compounds are decomposed by sulphuretted hydrogen and the guanine separated from the adenine and hypoxanthine by treatment while hot with ammonia, in which the guanine is difficultly soluble.

When the above filtrate containing the adenine and hypoxanthine, which has been, if necessary, freed from ammonia by evaporation, is allowed to cool, the adenine separates, while the hypoxanthine remains in solution. According to BALKE [1]—we can advantageously precipitate the purine bases with copper sulphate and hydroxylamine, following the method suggested by KRÜGER and SCHITTENHELM [2] for the quanti-

[1] See foot-note 3, page 184.

[2] Zeitschr. f. physiol. Chem., 45.

tative determination of the purines in feces. Details for the above methods may be found in complete hand-books. The same procedures are followed in the quantitative estimation of the purine bases in animal organs.[1]

3. Pyrimidine Bases.

These bodies are closely related to the purines, and pyrimidine,

$$C_4H_4N_2, = \begin{array}{l} N{=}CH \\ \;\;| \quad\;\; | \\ HC \quad CH \\ \;\;\| \quad\;\; \| \\ N{-}CH \end{array}$$

, may be considered as the mother substance thereof.

The pyrimidine bases contained in the nucleic acids are cytosine, uracil and thymine.

Cytosine, $C_4H_5N_3O, = \begin{array}{l} HN{-}CNH_2 \\ \;\;\| \quad\;\; \| \\ OC \quad CH \\ \;\;| \quad\;\; | \\ N{=}CH \end{array}$ (6 amino-2 oxypyrimidine), was first prepared by Kossel and Neumann from thymus nucleic acid, and then by Kossel and Steudel and others from various animal nucleic acids, and finally also by Wheeler and Johnson from triticonucleic acid. Wheeler and Johnson[2] have also prepared it synthetically. It is transformed into uracil by the action of nitrous acid.

The free base is difficultly soluble in water (129 parts) and crystallizes in thin leaves with a mother-of-pearl luster. It is insoluble in ether and difficultly soluble in alcohol. The double compound with platinum chloride, the crystalline picrate, the nitrate, and the two sulphates are of importance in the detection of cytosine. This base is precipitated by phosphotungstic acid and by silver nitrate in the presence of an excess of barium hydroxide, which fact is of importance in the detection of cytosine (Kutscher). The double bismuth-potassium iodide gives a brick-red precipitate. Cytosine gives the murexid reaction with chlorine-water and ammonia (see Chapter XV), and also the reaction described by Wheeler and Johnson under uracil. In regard to preparation see Kossel and Steudel[3] and also Kutscher.[4]

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[1] See Burian and Hall, Zeitschr. f. physiol. Chem., **38**; Kossel and Wulff, *ibid.*, **17**; Bruhns, *ibid.*, **14**; His and Hagen, *ibid.*, **30**.

[2] Amer. chem. Journ., **29**.

[3] Zeitschr. f. physiol. Chem., **37** and **38**.

[4] *Ibid.*, **38**. As it is not excluded, but rather probable according to Wheeler, that besides thymine also other related pyrimidine bases such as isocytosine, 6-amino pyrimidine and 6-oxpyrimidine can be formed in the hydrolytic cleavage of the nucleic acids, Wheeler has prepared salts and compounds of these bodies and described them as a matter of comparison, Journ. of biol. Chem., **3**.

Uracil, $C_4H_4N_2O_2, = \begin{array}{l} HN—CO \\ \;\;| \quad\;\; | \\ OC \quad CH \\ \;\;| \quad\;\; \| \\ HN—CH \end{array}$ (2, 6-dioxypyrimidine), was first obtained by ASCOLI and KOSSEL from yeast nucleic acid and later obtained from various complex nucleic acids, perhaps secondarily from the cytosine as a cleavage product. The synthetical preparation was first accomplished by E. FISCHER and ROEDER.[1]

Uracil crystallizes in needles which cluster in rosettes. On careful heating it sublimes in part undecomposed, but develops red vapors and decomposes in part. It is readily soluble in hot water, but less so in cold water, and nearly insoluble in alcohol and in ether. Uracil is readily soluble in ammonia. It is precipitated by mercuric nitrate, but not by phosphotungstic acid. It is precipitated by silver nitrate only on the careful addition of ammonia or baryta-water. Uracil gives the WEIDEL reaction and the following reaction described by WHEELER and JOHNSON:[2] The uracil solution is treated with bromine-water until it is permanently cloudy and then treated with baryta-water, when a purple or violet-colored precipitate appears almost immediately. The coloration varies with the dilution. This reaction which, as remarked above, is also given by cytosine, depends upon the fact that dibromoxyhydrouracil is first formed, and from this, by the action of the barium hydroxide, first isodialuric and then dialuric acid is produced, both of which give the coloration. In regard to the preparation of uracil see KOSSEL and STEUDEL.[3]

Thymine, $C_5H_6N_2O_2, = \begin{array}{l} HN—CO \\ \;\;| \quad\;\; | \\ OC \quad C.CH_3 \\ \;\;| \quad\;\; \| \\ HN—CH \end{array}$ (5-methyluracil). This body, which is identical with *nucleosin* obtained by SCHMIEDEBERG from salmo-nucleic acid, was first prepared by KOSSEL and NEUMANN from thymus-nucleic acid, and then by other investigators, especially LEVENE and MANDEL, from other animal nucleic acids. FISCHER and ROEDER and recently GERNGROSS[4] have prepared it synthetically.

Thymine crystallizes in small leaves grouped in stellar or dendriform

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[1] Ascoli, Zeischr. f. physiol. Chem., **31**; Kossel and Steudel, *ibid.*, **37**; Levene, *ibid.*, **38**, **39**; Levene and Mandel, *ibid.*, **49**; E. Fischer and Roeder, Ber. d. d. chem. Gesellsch., **34**.

[2] Journ. of biol. Chem., **3**.

[3] Zeitschr. f. physiol. Chem., **37**.

[4] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **37**; Kossel and Neumann, Ber. d. d. chem. Gesellsch., **26** and **27**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46**, **47**, **49**, **50**; E. Fischer and Roeder, *ibid.*, **34**; Gerngross, *ibid.*, **38**.

clusters or, rarely, in short needles (GULEWITSCH [1]). It melts at about 321° and sublimes. It is difficultly soluble in cold water, more soluble in hot water, and insoluble in alcohol. It behaves like uracil toward ammonia or baryta-water and silver nitrate. Thymine is precipitated by phosphotungstic acid, especially when impure. Bromine-water is decolorized by thymine, producing bromthymine. For its detection we make use of the sublimation, the behavior toward silver nitrate, and its elementary analysis.

In regard to the methods of preparation see KOSSEL and NEUMANN and W. JONES.[2]

The purine and pyrimidine bodies stand in close chemical and physiological relation to each other and for this reason the question has been repeatedly raised whether the pyrimidine bases might not be formed, at least in part, from the purine bases by the action of acids. Thus far no conclusive investigations have been made supporting this view, while on the contrary the investigations of STEUDEL [3] seem to contradict such a view.

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[1] Zeitschr. f. physiol. Chem., **27**.

[2] Kossel and Neumann, l. c., and W. Jones, Zeitschr. f. physiol. Chem., **29**, 461.

[3] Zeitschr. f. physiol. Chem., **51** and **53** (against Burian).

CHAPTER IV.

THE CARBOHYDRATES.

We designate by this name bodies which are especially abundant in the plant kingdom. As the protein bodies form the chief portion of the solids in animal tissues, so the carbohydrates form the chief portion of the dry substance of the plant structure. They occur in the animal kingdom only in proportionately small quantities, either free or in combinations with more complex molecules, forming compound proteins. Carbohydrates are of extraordinarily great importance as food for both man and animals.

The carbohydrates contain only *carbon, hydrogen,* and *oxygen.* The last two elements occur, as a rule, in the same proportion as they do in water, namely, 2:1, and this is the reason why the name carbohydrates has been given to them. This name is not quite pertinent, if strictly considered; because we not only have bodies, such as acetic acid and lactic acid, which are not carbohydrates and still have their oxygen and hydrogen in the same proportion as in water, but we also have a sugar (the methyl pentoses, $C_6H_{12}O_5$) which has these two elements in another proportion. At one time it was thought possible to characterize as carbohydrates those bodies which contained 6 atoms of carbon, or a multiple, in the molecule, but this is not considered tenable at the present time. We have true carbohydrates containing less than 6, and also those containing 7, 8, and 9 carbon atoms in the molecule.

The carbohydrates have no properties or characteristics in general which differentiate them from other bodies; on the contrary, the various carbohydrates are in many cases very different in their external properties. Under these circumstances it is very difficult to give a positive definition for the carbohydrates.

From a chemical standpoint we can say that all carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols. The simplest carbohydrates, the simple sugars or monosaccharides, are either aldehyde or ketone derivatives of such alcohols, and the more complex carbohydrates seem to be derived from these by the formation of anhydrides. It is a fact that the more complex carbohydrates yield two or even more molecules of the simple sugars when made to undergo hydrolytic splitting.

Correspondingly the carbohydrates can be divided into three chief groups, namely, 1. *Simple sugars* or monosaccharides, 2. *Complex sugars* or disaccharides, trisaccharides and crystalline polysaccharides, and 3. Non-crystalline or *colloid polysaccharides*. Of these groups the monosaccharides, disaccharides and colloid polysaccharides are of special physiological importance.

Our knowledge of the carbohydrates and their structural relationships has been very much extended by the pioneering investigations of KILLIANI [1] and especially those of E. FISCHER.[2]

As the carbohydrates occur chiefly in the plant kingdom it is naturally not the place here to give a complete discussion of the numerous carbohydrates known up to the present time. According to the plan of this work it is only possible to give a short review of those carbohydrates which occur in the animal kingdom or are of special importance as food for man and animals.

1. Monosaccharides.

All varieties of sugars are characterized by the termination "*ose*," to which a root is added signifying their origin or other relations. According to the number of carbon atoms, or more correctly oxygen atoms, contained in the molecule the monosaccharides are divided into, *trioses*, *tetroses*, *pentoses*, *hexoses*, *heptoses*, and so on.

All monosaccharides are either aldehydes or ketones of polyhydric alcohols. The former are termed *aldoses* and the latter *ketoses*. Ordinary dextrose is an aldose, while ordinary fruit sugar (levulose) is a ketose. The difference may be shown by the structural formulæ of these two varieties of sugar:

$$\text{Dextrose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO;$$
$$\text{Levulose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH).$$

A difference is also observed on oxidation. The aldoses can be converted into oxyacids having the same quantity of carbon, while the ketoses yield acids having less carbon. On mild oxidation the aldoses yield monobasic oxyacids and dibasic acids on more energetic oxidation. Thus ordinary dextrose yields gluconic acid in the first case and saccharic acid in the second.

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[1] Ber. d. deutsch. chem. Gesellsch., **18**, **19**, and **20**.

[2] See E. Fischer's lecture, Synthesen in der Zuckergruppe, Ber. d. deutsch. chem. Gesellsch., **23**, 2114. Excellent works on carbohydrates are Tollens' Kurzes Handbuch der Kohlehydrate, Breslau, **2**, 1895, and **1**, 2. Auflage, 1898, which gives a complete review of the literature, and E. O. v. Lippmann, Die Chemie der Zuckerarten, Braunschweig, 1904.

Gluconic acid $= CH_2(OH).[CH(OH)]_4.COOH$;
Saccharic acid $= COOH.[CH(OH)]_4.COOH$.

The monocarboxylic acids are easily transformed into their anhydrides (lactones), and these latter are of special interest because, as shown by FISCHER, they can be changed into the corresponding aldehyde, i.e. the corresponding aldose, by nascent hydrogen.

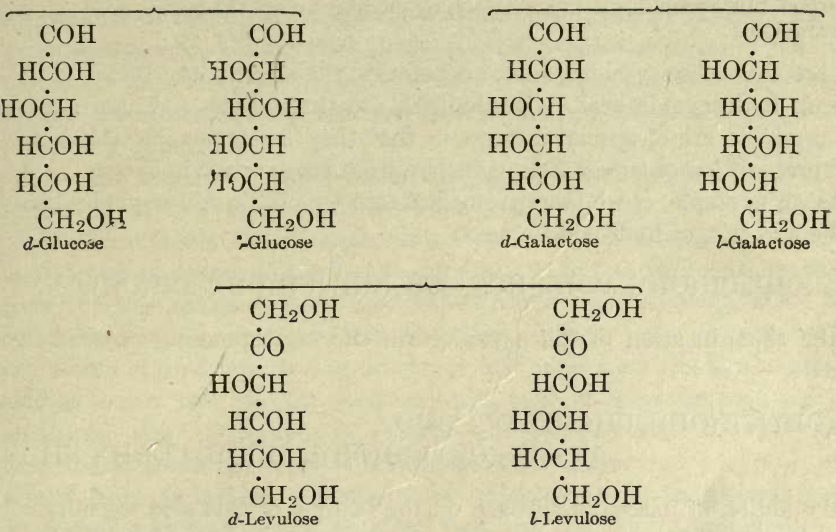
The monosaccharides are converted into the corresponding polyhydric alcohols by nascent hydrogen. Thus ARABINOSE, which is a pentose, $C_5H_{10}O_5$, is transformed into the pentatomic alcohol, ARABITE, $C_5H_{12}O_5$. The three hexoses, DEXTROSE, LEVULOSE, and GALACTOSE, $C_6H_{12}O_6$, are transformed into the corresponding three hexites, SORBITE, MANNITE, and DULCITE, $C_6H_{14}O_6$. The ketoses, on the contrary, due to their constitution, yield a mixture of two alcohols on the same treatment. From *d*-levulose for example we obtain a mixture of *d*-sorbite and *l*-mannite. On careful oxidation of the polyhydric alcohols the corresponding sugar can be prepared.

Numerous isomers occur among the monosaccharides, and especially in the hexose group. In certain cases, as, for instance, in glucose and levulose, we are dealing with a different constitution (aldoses and ketoses), but in most cases we have stereoisomerism due to the presence of asymmetric carbon atoms.

As the monosaccharides from the trioses upward contain asymmetric carbon atoms they occur as optically active bodies in a *l*-, *d*-, and racemic form, *r* or *d-l* form, which is a combination of the first two forms. As the number of asymmetric carbon atoms increases so does the number of possible stereoisomeric forms enlarge. As the number according to VAN'T HOFF is 2^n, where n represents the number of asymmetric carbon atoms, then for the aldo-hexose, which contains 4 asymmetric carbon atoms, $2^4 = 16$ stereo-chemically different forms can exist. In fact, of these, 12 have been prepared and their geometric structure has been explained and for which FISCHER has given configuration formulæ.

As these relations are readily conceived we will, for example, only give the configuration formulæ for the most important pentoses and hexoses occurring in the animal body.

COH	COH	COH	COH
HOCH	HCOH	HOCH	HCOH
HCOH	HOCH	HCOH	HOCH
HCOH	HOCH	HOCH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
d-Arabinose	*l*-Arabinose	*d*-Xylose	*l*-Xylose



We designate the optical activity of the carbohydrates with the letter *l-* for levogyrate, *d-* for dextrogyrate, and *r-* for the racemic. These are only partly indicative. Thus dextrorotatory glucose is designated *d*-glucose, levorotatory *l*-glucose, but EMIL FISCHER has used these signs in another sense. He designates by these signs the mutual relationship of the various kinds of sugars instead of their optical activity. For example, he does not designate the levorotatory levulose *l*-levulose, but *d*-levulose, showing its close relation to dextrorotatory *d*-glucose. This designation is generally accepted, and the above-mentioned signs only show the optical properties in certain cases.

Specific rotation means the rotation in degrees produced by 1 gm. substance dissolved in 1 cc. liquid placed in a tube 1 dcm. long. The reading is ordinarily made at 20° C. and with the monochromatic sodium light. The specific rotation with this light is represented by $(\alpha)_D$, and is expressed by the following formula: $(\alpha)_D = \pm\frac{a}{p.l}$, in which a represents the reading of degrees, l the length of the tube in decimeters, and p the weight of substance in 1 cc. of the liquid. Inversely the per cent P of substance can be calculated, when the specific rotation is known, by the formula $P = \frac{100a}{s.l}$, in which s represents the known specific rotation.

In the determination of the change in specific rotation with various concentrations we must know also the amount of substance in grams in 1 gram of the solution (p) and the specific gravity of the solution (d) at 20°. The rotation is calculated according to the formula $(\alpha)_D = \pm\frac{a}{p.l.d.}$.

A freshly prepared solution of a substance often shows a different rotation from one that has been allowed to stand for some time (multirotation). The correct values which are found on allowing the solution to stand for a sufficiently

long time can be obtained immediately by boiling or on the addition of very little ammonia.

Like the ordinary aldehydes and ketones, the sugars may be made to take up hydrocyanic acid. Cyanhydrins are thus formed. These addition products are of special interest in that they make possible the artificial preparation of sugars rich in carbon from sugars poor in carbon.

As an example, if we start from dextrose we obtain glucocyanhydrin on the addition of hydrocyanic acid:

$$CH_2.(OH).[CH(OH)]_4.COH + HCN = CH_2(OH).[CH(OH)]_4.CH(OH).CN.$$

On the saponification of glucocyanhydrin the corresponding oxyacid is formed:

$$CH_2(OH).[CH(OH)]_4.CH(OH).CN + 2H_2O = CH_2(OH).[CH(OH)]_4.CH(OH).COOH + NH_3.$$

By the action of nascent hydrogen on the lactone of this acid we obtain glucoheptose, $C_7H_{14}O_7$ and according to this principle the construction of sugars up to nine carbon atoms has been accomplished.

The monosaccharides give the corresponding oximes with hydroxylamine; thus glucose yields glucosoxime, $CH_2(OH).[CH(OH)]_4.CH:N.OH$. These compounds are of importance on account of the fact, as found by Wohl,[1] that they are the starting-point in the formation of one class of sugars from another class, namely, the preparation of sugars poor in carbon from those rich in carbon. For example pentoses from hexoses (see Wohl).

According to Ruff, by the action of hydrogen peroxide and the catalytic action of ferric salts upon the carbohydrate monocarboxylic acids the carbon chain can be shortened by the splitting off of the elements of formic acid, and with the formation of the next lower aldose. Neuberg[2] has accomplished the same result by electrolysis, and by this method has split glucose step by step into formaldehyde.

By the action of alkalies, even in small amounts, as also of carbonates and lead hydroxide, a reciprocal transformation of the sugars, such as *d*-dextrose, *d*-levulose, and *d*-mannose, may take place (Lobry de Bruyn and Alberda van Ekenstein[3]), and from each of these three varieties of sugar the two others are produced so that after a certain time the solution contains all three sugars.

The transformation of the different varieties of sugar into each other

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[1] Ber. d. d. chem. Gesellsch., 26, p. 730.

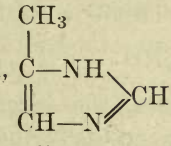
[2] Ruff, Ber. d. d. chem. Gesellsch., 31 and 32; Neuberg, Biochem. Zeitschr., 7.

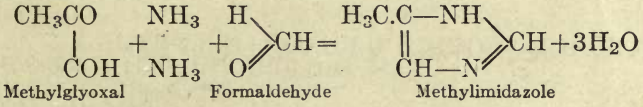
[3] Ber. d. d. chem. Gesellsch., 28, 3078; Bull. soc. chim. de Paris (3), 15; Chem. Centralbl., 1896, 2, and 1897, 2.

also occurs in the animal body. NEUBERG and MAYER [1] have shown by experiments on rabbits the direct partial transformation of various mannoses into the corresponding glucoses. Another example is, it seems, the formation of galactose (see milk sugar) from glucose in the mammary gland.

By the action of strong alkali the sugars are decomposed with the formation of lactic acid and many other products.

With ammonia the glucoses may form compounds which have been considered as osamines by LOBRY DE BRUYN, but to differentiate them from the true osamines have been called osimines by E. FISCHER.[2] The corresponding osaminic acid can be obtained from such an osimine by the action of ammonia and hydrocyanic acid, and from the hydrochloric-acid lactone of this acid the osamine is obtained by reduction with sodium amalgam. In this manner E. FISCHER and LEUCHS artificially prepared first *d*-arabinosimine from *d*-arabinose, then *d*-glucosaminic acid and finally from its lactone *d*-glucosamine, which occurs in the animal body. In a similar manner they [3] obtained *l*-glucosamine from *l*-arabinose.

KNOOP and WINDAUS [4] have obtained large amounts of methylimidazol,  , from glucose by the action of ammonium-zinc hydroxide at ordinary temperatures. This formation can be conceived as follows: First methyl glyoxal is formed from the sugar, and then from this or from the sugar formaldehyde is produced, which reacts with the methyl glyoxal with the formation of methylimidazole according to the following equation:



A genetic relationship of the carbohydrates to histidine and the purine bodies is thus made probable by the imidazole formation.

As the sugars are derivatives of polyhydric alcohols, they also form esters, among which the benzoyl ester is of special interest because it is used in the detection and isolation of the sugars and also of other carbohydrates. The nucleic acids probably also belong to the acid esters of the sugars, and thus may be considered as complex phosphoric acid

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[1] Zeitschr. f. physiol. Chem., **37**.

[2] Lobry de Bruyn, Ber. d. d. chem. Gesellsch., **28**; E. Fischer, *ibid.*, **35**.

[3] *Ibid.*, **36** and **35**, p. 3787.

[4] *Ibid.*, **38**, and Hofmeister's Beiträge, **6**.

esters, and perhaps the chondroitin sulphuric acid and the glucothionic acid are sulphuric acid esters. The nature of these two groups of sulphuric acid esters is not yet thoroughly understood.

The sugars can also combine with other bodies and with each other, forming ether-like combinations. By the action of hydrochloric acid as catalyst, as shown by FISCHER and collaborators, the sugars split off water and unite with other bodies, producing lactone-like combinations, which have been called *glucosides*. These glucosides, which are generally compounds with aromatic groups, occur widely distributed in the vegetable kingdom. The more complex carbohydrates may be considered, according to FISCHER, as glucosides of the sugars. Thus maltose, for example, is the glucoside and lactose the galactoside of dextrose. The glucosides can be split into their components by chemical agents, boiling with dilute mineral acids, as well as by the action of enzymes. The complex sugars hereby yield simple sugars and the other glucosides yield compounds which belong either to the aromatic or the aliphatic series besides the sugar. A long-known example of a decomposition of this kind is the splitting of amygdalin by the enzyme emulsin. It yields glucose, benzaldehyde and hydrocyanic acid according to the equation:

$$C_{20}H_{27}NO_{11}+2H_2O=2C_6H_{12}O_6+C_6H_5COH+CNH.$$

With phenylhydrazine or substituted phenylhydrazines, the sugars first yield *hydrazones* with the elimination of water, and then on the further action of hydrazine on warming in an acetic-acid solution we obtain *osazones*.

The reaction takes place with the aldoses as follows:

(*a*) $CH_2(OH).[CH(OH)]_3.CH(OH).CHO+H_2N.NH.C_6H_5=$
$CH_2(OH).[CH(OH)]_3.CH(OH)CH:N.NH.C_6H_5+H_2O.$
Phenylglucosehydrazone

(*b*) $CH_2(OH)[CH(OH)]_3.CH(OH).CH:N.NH.C_6H_5+H_2N.NH.C_6H_5=$
$CH_2(OH).[CH(OH)]_3.C.CH:N.NH.C_6H_5$
$\ddot{N}.NH.C_6H_5+H_2O+H_2.$
Phenylglucosazone

and with the ketoses:

$$CH_2(OH)[CH(OH)]_3CO.CH_2(OH)+H_2N.NH.C_6H_5=$$
$$CH_2(OH)[CH(OH)]_3\ddot{C}.CH_2OH$$
$$\ddot{N}.NH.C_6H_5+H_2O,$$

and $CH_2(OH)[CH(OH)]_3.\ddot{C}.CH_2(OH)$
$\ddot{N}.NH.C_6H_5+H_2N.NH.C_6H_5=$

$CH_2(OH)[CH(OH)]_3.C.CH:N.NH.C_6H_5 \quad +H_2O+H_2.$
$\ddot{N}.NH.C_6H_5$

The hydrogen is not evolved, but acts on a second molecule of phenylhydrazine and splits it into aniline and ammonia:

$$H_2N.H.C_6H_5+H_2=H_2N.C_6H_5+NH_3.$$

For the isolation of the sugars we often make use of the hydrazones as well as the osazones obtained by the aid of substituted phenyl hydrazines instead of the phenyl hydrazine, because they yield difficultly soluble compounds. As seen from the above equations the aldoses and ketoses yield the same osazones, while the hydrazones are different.

The osazones, which are more important than the hydrazones, are generally yellow crystalline compounds which differ from each other in melting-point, solubility, and optical properties, and hence have been of great importance in the characterization of certain sugars. They have also become of extraordinarily great interest in the study of the carbohydrates for other reasons. Thus they are a very good means of precipitating sugars from solution in which they occur mixed with other bodies, and they are of the greatest importance in the artificial preparation of sugars. On cleavage, by the brief action of gentle heat and fuming hydrochloric acid (for disaccharides still better with benzaldehyde),[1] the osazones yield so-called *osones*, which on reduction yield aldoses or more often ketoses.

We can also pass from the osazones to the corresponding sugars in other, indirect, ways. The hydrazones can be much more readily retransformed into the corresponding sugar, especially by decomposition with benzaldehyde (Herzfeld) or with formaldehyde (Ruff and Ollendorff[2]), whereby the sugar is replaced by the aldehyde used.

An important property, although not applicable to all sugars, is their ability to undergo *fermentation*, especially their ability to undergo alcoholic fermentation with alcohol-yeast. We must state, however, that the power of fermentation with pure yeast has been shown only for the hexose group, and in fact all of the hexoses do not ferment, and they do not all ferment with the same readiness. *d*-Glucose and *d*-mannose ferment readily, but *d*-galactose only with difficulty. The *l*-forms of the above-mentioned sugars do not ferment, and from the racemic forms of these sugars the optical *l*-antipode can be prepared by the fermentation of the *d*-sugar. Among the ketoses the *d*-levulose ferments while the sorbose does not. Among the sugars containing nine atoms of carbon, the nonoses, the mannonose does not ferment while the gluconónose does. The different behavior of the various sugars with yeast stands in fixed relation to their configuration, and is not only of great importance for the behavior of the sugar in lower organisms, but also for their behavior in higher developed organisms. Thus the investigations of Neuberg and Wohlgemuth[3] upon arabinose and of Neuberg and Mayer[4] on man-

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[1] E. Fischer and Armstrong, Ber. d. d. chem. Gesellsch., **35**.

[2] Herzfeld, *ibid.*, **28**; Ruff and Ollendorff, *ibid.*, **32**.

[3] Zeitschr. f. physiol. Chem., **35**.

[4] *Ibid.*, **37**.

noses have shown that in rabbits the *l*-arabinose and the *d*-mannose are much better utilized than *d*- and *r*-arabinose or *l*- and *r*-mannose. These observations as well as the varying fermentability of the sugars are only a few examples showing the great influence that chemical structure and configuration have upon chemical compounds in making the material changes in the animal and plant kingdom.

In the alcoholic fermentation the sugar is decomposed according to the general equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. The exact process is not clear, and seems to be rather complicated. According to the investigations of BUCHNER and MEISSENHEIMER, STOKALSA and MAZÉ, [1] we are dealing here with the complex action of two enzymes, of which one, the *zymase* (BUCHNER and MEISSENHEIMER), transforms the sugar into lactic acid, while the other, the *lactacidase* (BUCHNER and MEISSENHEIMER) splits the lactic acid into alcohol and carbon dioxide. According to certain investigators methyl glyoxal, $CH_3CO.COH$, is formed intermediary between the sugar and the lactic acid.[2]

As previously mentioned, the sugars also undergo other fermentations, namely lactic acid and butyric acid fermentation.

The monosaccharides are colorless and odorless bodies, neutral in reaction, with a sweet taste, readily soluble in water, generally soluble with difficulty in absolute alcohol, and insoluble in ether. Some of them crystallize well in the pure state. They are strong reducing substances. They reduce metallic silver from ammoniacal silver solutions and they also reduce other metallic oxides such as copper, bismuth and mercury oxides, on heating in alkaline solution. This behavior is of great importance in the detection and quantitative estimation of the sugars.

The simple varieties of sugar occur in part in nature as such, already formed, which is the case with both of the very important sugars, dextrose and levulose. They also occur in great abundance in nature as more complex carbohydrates (di- and polysaccharides); also as ester-like combinations with different substances, as so-called glucosides.

Among the groups of monosaccharides known at the present time, those containing less than five and more than six carbon atoms in the molecule have no great importance in biochemistry, although they are of high scientific interest. Of the two groups the hexoses are the more abundant and are of special interest. The pentoses are becoming of

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[1] Buchner and Meissenheimer, Ber. d. d. chem. Gesellsch., **37** and **38**; Stoklasa, Ber. d. d. Botan. Gesellsch., **22**, pages 358 and 460; Mazé, Compt. rend., **138**.

[2] Buchner and Meissenheimer, l. c. and Ber. d. d. chem. Gesellsch., **39**. In regard to the chemical processes in alcoholic fermentation see also Schade, Zeitschr. f. physikal. Chem., **57**, and Biochem. Zeitschr., **7**; Wohl, *ibid.*, **5**; Slator, Ber. d. d. chem. Gesellsch., **40**.

greater importance, not only for the chemistry of plants, but also for the chemical processes in the animal body.

Pentoses ($C_5H_{10}O_5$).

As a rule the pentoses do not occur as such in nature. They are obtained from animal tissues, organs and fluids as cleavage products of the nucleic acids or nucleoproteins. The pentoses are chiefly obtained from the plant kingdom by the hydrolytic cleavage with dilute mineral acids, of more complex carbohydrates, the so-called pentosans. The *pentosans* exist very widely distributed in the plant kingdom, and are of especially great importance in the building up of certain plant constituents. Methyl pentosans and methyl pentoses also occur in the plants, and of these, the methyl pentose, rhamnose, which occurs in several glucosides, must be specially mentioned.

The pentoses were first found in the animal kingdom by Salkowski and Jastrowitz in the urine of a person addicted to the morphine habit, and later by Salkowski and others in human urine. Small quantities of pentoses have been detected by Külz and Vogel[1] in the urine of diabetics, as also in dogs with pancreas diabetes or phlorhizin diabetes. Pentose has also been found by Hammarsten among the cleavage products of a nucleoprotein obtained from the pancreas, or from the corresponding guanylic acid, and seems also, according to the observations of Blumenthal, to be a constituent of nucleoproteins of various organs, such as the thymus, thyroid, brain, spleen, and liver. In regard to the quantity of pentoses found in the various organs, we must refer to the works of Grund and of Bendix and Ebstein and Mancini.[2]

The pentosans (Stone, Slowtzoff) as well as the pentoses are of the greatest importance as foods for herbivorous animals. In regard to the value of the pentoses, the researches of Salkowski, Cremer, Neuberg, and Wohlgemuth[3] upon rabbits and hens show that these animals can utilize the pentoses. The question whether the pentoses are active as glycogen-formers is still an open one (see Chapter VIII). The pen-

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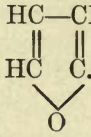
[1] Salkowski and Jastrowitz. Centralbl. f. d. med. Wissensch., 1892, 337 and 593; Salkowski, Berl. klin. Wochenschr., 1895; Bial, Zeitschr. f. klin. Med., **39**; Bial and Blumenthal, Deutsch. med. Wochenschr., 1901, No. 2; Külz and Vogel, Zeitschr. f. Biologie, **32**.

[2] Hammarsten Zeitschr. f. physiol. Chem., **19**; also Salkowski, Berl. klin. Wochenschr., 1895; Blumenthal, Zeitschr. f. klin. Med., **34**; Grund, Zeitschr. f. physiol. Chem., **35**; Bendix and Ebstein, Zeitschr. f. allgemein. Physiol., **2**; Mancini, Chem. Centralbl., 1906.

[3] Stone, Amer. Chem. Journ., **14**; Slowtzoff, Zeitschr. f. physiol. Chem., **34**; Salkowski, *ibid.* **32**; Cremer, Zeitschr. f. Biologie, **29** and **42**; Neuberg and Wohlgemuth, Zeitschr. f. physiol. Chem., **35**.

toses seem to be absorbed by human beings and in part utilized, but they pass in part into the urine even when small quantities are taken.[1]

The natural pentoses are reducing aldoses, and as a rule do not belong to the sugars fermentable by yeast. Still, the observations of Salkowski, Bendix, Schöne and Tollens seem to indicate that the pentoses are fermentable[2]. They are readily decomposed by putrefaction bacteria. With phenylhydrazine and acetic acid they give crystalline osazones which are soluble in hot water, and whose melting-points and optical behavior are important for the detection of the pentoses. On heating with hydrochloric acid they yield furfurol, but no levulinic acid. In this reaction furfuran is formed from the pentose molecule, and then from this its adehyde, the furfurol  The furfurol passing over on distilling with hydrochloric acid can be detected by the aid of aniline-acetate paper, which is colored beautifully red by furfurol. In the quantitative estimation we can use the method suggested by Tollens, which consists in converting the furfurol in the distillate into phloroglucide by means of phloroglucin and weighing (see Tollens and Kröber, Grund, Bendix and Ebstein), or according to Jolles[3] by bisulphite and retitrating with iodine solution. In using these methods it must be borne in mind that glucuronic-acid compounds also yield furfurol under the same conditions. The two following pentose reactions, as suggested by Tollens, are especially applicable.

The **orcin-hydrochloric acid test.** Mix with the solution or the substance introduced into water an equal volume of concentrated hydrochloric acid, add some orcin in substance, and heat. In the presence of pentoses the solution becomes reddish blue, then bluish green, and on spectroscopic examination an absorption-band is observed between *C* and *D*. If it is cooled and shaken with amyl alcohol, a bluish-green solution which shows the same band is obtained.

The **phloroglucin-hydrochloric acid test.** This test is performed in the same manner as the above, using phloroglucin instead of orcin. The solution becomes cherry-red on heating and then becomes cloudy and hence a shaking out with amyl alcohol is very necessary. The red amyl-

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[1] See Ebstein, Virchow's Arch., **129**; Tollens, Ber. d. deutsch. chem. Gesellsch., **29**, 1208; Cremer, l. c.; Lindemann and May, Deutsch. Arch. f. klin. Med., **56**; Salkowski, Zeitschr. f. physiol. Chem., **30**.

[2] Salkowski, Zeitschr. f. physiol. Chem., **30**; Bendix, see Chem. Centralbl., 1900, **1**; Schöne and Tollens, *ibid.*, 1901, **1**.

[3] Bendix and Ebstein, l. c., which contains the literature; Jolles, Ber. d. d. chem. Gesellsch., **39** and Zeitschr. f. anal. Chem., **46**.

alcohol solution shows an absorption-band between *D* and *E*. The orcin test is better for several reasons than the phloroglucin test (SALKOWSKI and NEUBERG [1]). In regard to the use of these tests in urine examination see Chapter XV.

Many modifications of these tests have been suggested. BRAT [2] performs the orcin reaction by the addition of NaCl and heating to only 90–95°. BIAL [3] uses a hydrochloric acid containing ferric chloride for the orcin test and claims to get a greater delicacy. On too strong and too long heating (1½–2 minutes), when using this modification, a confusion with sugars of the six carbon series may occur (BIAL, VAN LEERSUM).[4] According to R. ADLER and O. ADLER the phloroglucin and orcin tests can be made with glacial acetic acid and a few drops hydrochloric acid instead of with the hydrochloric acid alone. These investigators also use a mixture of equal volumes of aniline and glacial acetic acid as a reagent for pentoses. On the addition of a little pentose to the boiling mixture a beautiful red color of furfurol-aniline acetate is obtained. A. NEUMANN [5] performs the orcin test with glacial acetic acid and adds concentrated sulphuric acid drop by drop. On following the exact instructions not only do the pentoses give this reaction, but also glucuronic acid, dextrose, and levulose give characteristic colored solutions with special absorption-bands which can be made use of in identifying the various sugars. FR. SACHS has tested BIAL'S test and has given special precautions to prevent confusion with glucuronic acid. JOLLES [6] precipitates (from urine) the pentoses as osazones, distills the precipitate with hydrochloric acid, and tests the distillate with BIAL'S reagent.

In performing the above two tests for pentose it must be borne in mind that glucuronic acid gives the same reactions and also that the colors alone are not sufficient. The spectroscopic examination must therefore never be omitted. Both tests are to be considered as tests of detection rather than definite pentose reactions, and therefore for a positive detection of pentoses we must prepare also the osazones or other compounds.

Arabinoses. The pentose isolated by NEUBERG from human urine is *r*-arabinose. It can be isolated from the urine as the diphenylhydrazone, from which the arabinose can be separated by splitting with formaldehyde. The inactive *r*-arabinose seems to be the pentose regularly occurring in pentosuria and thus far, in only one case, has *l*-arabinose been found (R. LUZZATTO). *l*-Arabinose is said to pass into the urine after partaking of certain fruits such as plums in large amounts (C. BARSZCZEWSKI [7]).

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[1] Salkowski, Zeitschr. f. physiol. Chem., **27**; Neuberg, *ibid.*, **31**.

[2] Zeitschr. f. klin. Med., **47**.

[3] Deutsch. med. Wochenschr., 1902 and 1903, and Zeitschr. f. klin. Med., **50**.

[4] Bial, Zeitschr. f. klin. Med., **50**; van Leersum, Hofmeister's Beiträge, **5**.

[5] R. and O. Adler, Pflüger's Arch., **106**; A. Neumann, Berl. klin. Wochenschr., 1904.

[6] Fr. Sachs, Biochem. Zeitschr., **1** and **2**; Jolles, *ibid.*, **2**, Centralbl. f. klin. Med., 1907, and Zeitschr. f. anal. Chem., **46**.

[7] Neuberg, Ber. d. d. chem. Gesellsch., **33**; Luzzatto, Hofmeister's Beiträge, **6**,

The *r*-arabinose is crystalline, has a sweetish taste, and melts at 163–164° C. Its diphenylhydrazone, which, according to NEUBERG and WOHLGEMUTH,[1] can be used in its quantitative estimation, melts at 206° C., is insoluble in cold water and alcohol, but readily soluble in pyridine. The osazone melts at 166–168° C.

The dextrorotatory *l*-arabinose is obtained by boiling gum arabic or cherry gum with dilute sulphuric acid. The *d*-arabinose has been prepared synthetically. The phenylozasone of *l*-arabinose melts at 160°. The *l*-arabinose which crystallizes in plates or prisms melts at about 164°. The specific rotation is $(\alpha)_D = +104.5°$.

Xyloses. The first pentose to be isolated from organs was *l*-xylose by NEUBERG from the pancreas neucleoproteins. NEUBERG and BRAHN claim to have isolated *l*-xylose from inosinic acid, but the investigations of LEVENE and JACOBS [2] show that the pentose in this case is not *l*-xylose, but rather *d*-ribose. *l*-Xylose occurs very extensively in the plant kingdom, and is obtained from wood-gums by boiling with dilute acids. Xylose is crystalline, melts at 150–153° C., dissolves very readily in water but with difficulty in alcohol, is faintly dextrorotatory, $(\alpha)_D = +18.1°$, and gives a phenylosazone which melts at 155–158° C., and according to TOLLENS and MÜTHER a diphenylhydrazone which melts at 107–108°. According to BERTRAND [3] xylose can be transformed into xylonic acid, $CH_2(OH)[CH(OH)]_3COOH$, by bromine-water and the brom-cadmium compound or the brucine salt (NEUBERG) of this acid is well suited for the detection and isolation of *l*-xylose.

Hexoses ($C_6H_{12}O_6$).

The most important and best-known simple sugars belong to this group, and most of the other bodies which have been considered as carbohydrates in the past are anhydrides of this group. Certain hexoses, such as dextrose and levulose, either occur in nature already formed or are produced by the hydrolytic splitting of other more complicated carbohydrates or glucosides. Others, such as mannose or galactose, are formed by the hydrolytic cleavage of other natural products, while some, on the contrary, such as gulose, talose, and others, are obtained only by artificial means.

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and Arch. f. exp. Path. u. Pharm., 1908, Schmiedeberg-Festschr.; Barszczewski, Maly's Jahrb., **27**, p 733.

[1] Zeitschr. f. physiol. Chem., **35**.

[2] Neuberg, Ber. d. d. chem. Gesellsch., **35**; Neuberg and Brahn, Biochem. Zeitschr., **5**, and Ber. d. d. chem. Gesellsch., **41**, p. 3376; Levene and Jacobs, Ber. d. d. chem. Gesellsch., **41** and **42**.

[3] Tollens and Müther, Ber. d. d. chem. Gesellsch., **37**; Bertrand, Bull. soc. chim. (3), **5**.

All hexoses, as also their anhydrides, yield levulinic acid, $C_6H_8O_3$, besides formic acid and humus substances on boiling with dilute mineral acids. Some of the hexoses, as above stated, are fermentable with yeast.

Some hexoses are aldoses, while others are ketoses. Belonging to the first group we have MANNOSE, DEXTROSE, and GALACTOSE, and to the other LEVULOSE, and possibly also SORBINOSE.

The most important syntheses of the carbohydrates have been made by E. FISCHER and his pupils, chiefly within the members of the hexose group. A short summary of the syntheses of hexoses is given below.

The first artificial preparation of a sugar was made by BUTLEROW. On treating trioxymethylene, a polymer of formaldehyde, with lime-water he obtained a faintly sweetish syrup called *methylenitan*. LOEW[1] later obtained a mixture of several sugars, from which he isolated a fermentable sugar, called *methose*, by condensation of formaldehyde in the presence of bases. The most important and comprehensive syntheses of sugar have been performed by E. FISCHER.[2]

The starting-point of these syntheses is *α-acrose*, which occurs as a condensation product of formaldehyde. The name α-acrose has been given to this body because it is obtained from acrolein bromide by the action of bases (FISCHER). It is also obtained admixed with *β-acrose* on the oxidation of glycerin with bromine in the presence of sodium carbonate and treating the resulting mixture with alkali. On the oxidation with bromine a mixture of glyceric aldehyde, $CH_2OH.CH(OH).CHO$, and dioxyacetone, $CH_2(OH).CO.CH_2OH$, is obtained. These two bodies may be considered as true sugars, namely, glyceroses or trioses. It seems as if a condensation to hexoses takes place on treatment with alkalies.

α-acrose may be isolated from the above mixture and obtained pure by first converting it into osazone and then retransforming this into the sugar. α-acrose seems to be identical with *r*-levulose. With yeast one half, the levogyrate *d*-levulose ferments, while the dextrogyrate *l*-levulose remains. The *r*- and *l*-levulose may be prepared in this way.

On the reduction of α-acrose we obtain α-acrite, which is identical with *r*-mannite. On oxidation of *r*-mannite we obtain *r*-mannose, from which only *l*-mannose remains on fermentation. On further oxidation of *r*-mannose it yields *r*-mannonic acid. The two active mannonic acids may be separated from each other by the fractional crystallization of their strychnine or morphine salts. The two corresponding mannoses may be obtained from these two acids, *d*- and *l*-mannonic acids, by reduction.

d-Levulose is obtained from *d*-mannose by the method given on page 196, using the osazone as an intermediate step. The *d*- and *l*-mannonic acids are partly converted into *d*- and *l*-gluconic acids on heating with quinoline, and *d*- or *l*-glucose is obtained on the reduction of these acids; *l*-glucose is best prepared from *l*-arabinose by means of the cyanhydrin reaction, using *l*-gluconic acid as the intermediate step. The combination of *l*- and *d*-gluconic acids, forming *r*-gluconic acid, yields *r*-glucose on reduction.

The artificial preparation of sugars by means of the condensation of formaldehyde has received special interest because, according to BAEYER'S assimilation hypothesis, in plants formaldehyde is first formed by the reduction of carbon dioxide, and the sugars are produced by the condensation of this formaldehyde.

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[1] Butlerow, Ann. d. Chem. u. Pharm., **102**; Compt. rend., **53**; O. Loew, Journ. f. prakt. Chem. (N. F.), **33**, and Ber. d. deutsch. chem. Gesellsch., **20**, **21**, **22**.

[2] Ber. d. d. chem. Gesellsch., **21**, and l. c., p. 193.

Bokorny [1] has shown, by special experiments on algæ Spirogyra, that formaldehyde sodium sulphite was split by the living algæ cells. The formaldehyde set free is immediately condensed to carbohydrate and precipitated as starch.

Among the hexoses known at the present time only dextrose, levulose and galactose are really of physiological-chemical interest; therefore of the other hexoses only mannose will be incidentally mentioned.

Dextrose (*d*-glucose)—GLUCOSE, GRAPE-SUGAR, and DIABETIC SUGAR—occurs abundantly in the grape, and also, often accompanied with levulose (*d*-fructose), in honey, sweet fruits, seeds, roots, etc. It occurs in the human and animal intestinal tract during digestion, also in small quantities in the blood and lymph, and as traces in other animal fluids and tissues. It occurs only as traces in urine under normal conditions, while in diabetes the quantity is very large. It is formed in the hydrolytic cleavage of starch, dextrin, and other compound carbohydrates, as also in the splitting of glucosides. The question whether dextrose can be formed in the body from proteins or from fats is disputed and will be discussed in a following chapter (VIII).

Properties of Dextrose. Dextrose crystallizes sometimes with 1 molecule of water of crystallization in warty masses consisting of small leaves or plates, and sometimes when free from water in fine needles or prisms. The sugar containing water of crystallization melts even below 100° C. and loses its water of crystallization at 110° C. The anhydrous sugar melts at 146° C., and is converted into glucosan, $C_6H_{10}O_5$, at 170° C. with the elimination of water. On strongly heating it is converted into caramel and then decomposes.

Dextrose is readily soluble in water. This solution, which is not as sweet as a cane-sugar solution of the same strength, is dextrogyrate and shows strong birotation. The specific rotation is dependent upon the concentration of the solution, as it increases with an increase in the concentration. A 10 per cent solution of anhydrous glucose can be taken as +52.5° at 20° C.[2] Dextrose dissolves sparingly in cold, but more freely in boiling alcohol. 100 parts alcohol of sp. gr. 0.837 dissolves 1.95 parts anhydrous dextrose at 17.5° C. and 27.7 parts at the boiling temperature (ANTHON [3]). Dextrose is insoluble in ether.

If an alcoholic caustic-potash solution is added to an alcoholic solution of dextrose, an amphorous precipitate of insoluble sugar-potash compound is formed. On warming this compound it decomposes easily with the formation of a yellow or brownish color, which is the basis of MOORE'S test. Dextrose also forms compounds with lime and baryta.

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[1] Biolog. Centralbl., **12**, pp. 321 and 481.

[2] For further information see Tollens, Handbuch der Kohlehydrate, 2. Aufl., 44.

[3] Cited from Tollens' Handbuch.

MOORE'S *Test.* If a dextrose solution is treated with about one quarter of its volume of caustic potash or soda and warmed, the solution becomes first yellow, then orange, yellowish brown, and lastly dark brown. It has at the same time a faint odor of caramel, and this odor is more pronounced on acidification.[1]

Dextrose forms several crystallizable combinations with NaCl, of which the easiest to obtain is $(C_6H_{12}O_6)_2.NaCl+H_2O$, which forms large colorless six-sided double pyramids or rhomboids with 13.52 per cent NaCl.

Dextrose in neutral or very faintly acid (organic acid) solution undergoes alcoholic fermentation with beer-yeast: $C_6H_{12}O_6=2C_2H_5.OH+2CO_2$. In the presence of acid milk or cheese the dextrose undergoes lactic-acid fermentation, especially in the presence of a base such as ZnO or $CaCO_3$. The lactic acid may then further undergo butyric-acid fermentation: $2C_3H_6O_3=C_4H_8O_2+2CO_2+4H$.

Dextrose reduces several metallic oxides, such as copper, bismuth, and mercuric oxide, in alkaline solutions, and the most important reactions for sugar are based on this fact.[2]

TROMMER'S test is based on the property that dextrose possesses of reducing cupric hydroxide in alkaline solution into cuprous oxide. Treat the dextrose solution with about $\frac{1}{5}$–$\frac{1}{8}$ vol. caustic soda and then carefully add a dilute copper-sulphate solution. The cupric hydroxide is thereby dissolved, forming a beautiful blue solution, and the addition of copper sulphate is continued until a very small amount of hydroxide remains undissolved in the liquid. This is now warmed, and a yellow hydrated suboxide or red suboxide separates even below the boiling temperature. If too little copper salt has been added, the test will be yellowish brown in color, as in MOORE'S test; but if an excess of copper salt has been added, the excess of hydroxide is converted on boiling into a dark-brown hydrate which interferes with the test. To prevent these difficulties the so-called FEHLING'S solution may be employed. This solution is obtained by mixing just before use equal volumes of an alkaline solution of Rochelle salt and a copper-sulphate solution (173 grams Rochelle salt and about 50–60 grams NaOH per liter and 34.65 grams crystalline copper sulphate per liter.) This solution is not reduced or noticeably changed by boiling. The tartrate holds the excess of cupric hydroxide in solution, and an excess of the reagent does not interfere in the performance of the test. In the presence of sugar this solution is reduced.

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[1] In regard to the products formed in this reaction, see Framm, Pflüger's Arch., **64**; Neff, Annal. d. Chem. u. Pharm., **357**; Buchner and Meissenheimer, Ber. d. d. chem. Gesellsch., **39**; Meissenheimer, *ibid.*, **41**.

[2] In regard to the products produced see Neff, Annal. d. Chem. u. Pharm., **357**.

According to BENEDICT[1] this test is more delicate if sodium carbonate is used instead of sodium hydroxide in the preparation of FEHLING'S solution.

BÖTTGER-ALMÉN'S test is based on the property dextrose possesses of reducing bismuth oxide in alkaline solution. The reagent best adapted for this purpose is obtained, according to NYLANDER'S[2] modification of ALMÉN'S original test, by dissolving 4 grams of Rochelle salt in 100 parts of 10 per cent caustic-soda solution and adding 2 grams of bismuth subnitrate and digesting on the water-bath until as much of the bismuth salt is dissolved as possible. If a dextrose solution is treated with about $\frac{1}{10}$ vol., or with a larger quantity of the solution when large quantities of sugar are present, and boiled for a few minutes, the solution becomes first yellow, then yellowish brown, and finally nearly black, and after a time a black deposit of bismuth (?) settles.

The property that dextrose has of reducing an alkaline solution of mercury on boiling is the basis of KNAPP'S reaction with alkaline mercuric cyanide and of SACHSSE'S reaction with an alkaline potassium-mercuric iodide solution.

On heating with PHENYLHYDRAZINE ACETATE a dextrose solution gives a precipitate consisting of fine yellow crystalline needles which are nearly insoluble in water, but soluble in boiling alcohol, and which separate again on treating the alcoholic solution with water. The crystalline precipitate consists of *phenylglucosazone* (see page 198). This compounds melts when pure at 204–205° C. It must be borne in mind that the melting-point of this and other osazones is somewhat variable, depending upon the rapidity of the heating, the diameter of the tube and the thickness of the sides of the tube.[3] The osazone dissolves readily in pyridine (0.25 gram in 1 gram), and precipitates again from this solution as crystals on the addition of benzene, ligroin, or ether. According to NEUBERG[4] this behavior can be used in the purification of the osazone. The diphenylhydrazone and the methyl phenylhydrazone are also of interest.

Dextrose is not precipitated by a lead-acetate solution, but is almost completely precipitated by a solution of ammoniacal basic lead acetate. On warming, the precipitate becomes flesh-color or rose-red (RUBNER'S *reaction*[5]).

If a watery solution of dextrose is treated with *benzoylchloride* and an excess of caustic soda, and shaken until the odor of benzoylchloride

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[1] Journ. of biol. Chem., 3.
[2] Zeitschr. f. physiol. Chem., 8.
[3] See E. Fischer, Ber. d. d. chem. Gesellsch., 41.
[4] Ber. d. d. chem. Gesellsch., 32, 3384.
[5] Zeitschr. f. Biologie, 20.

has disappeared, a precipitate of benzoic-acid ester of dextrose will be produced which is insoluble in water or alkali (BAUMANN [1]).

If ½–1 cc. of a dilute watery solution of dextrose is treated with a few drops of a 10 per cent alcoholic solution (free from acetone) of *α-naphthol*, the liquid is colored a beautiful violet on the addition of 1–2 cc. of concentrated sulphuric acid (MOLISCH). According to REINBOLD [2] this reaction depends first upon the formation of a volatile substance which gives a bluish-violet color with α-naphthol and sulphuric acid in the warmth. On further heating furfurol is also produced, which gives a raspberry-red to ruby-red coloration.

DIAZOBENZENESULPHONIC ACID gives with a dextrose solution made alkaline with a fixed alkali a red color, which after 10–15 minutes gradually changes to violet. ORTHONITROPHENYLPROPIOLIC ACID yields indigo when boiled with a small quantity of dextrose and sodium carbonate, and this is converted into indigo-white by an excess of sugar. An alkaline solution of dextrose is colored deep red on being warmed with a dilute solution of PICRIC ACID. The behavior of dextrose toward certain pentose reactions has already been given on page 203.

A more complete description as to the performance of these several tests will be given in detail in a subsequent chapter (on the urine).

Dextrose is prepared pure by inverting cane-sugar by the following simple method of SOXHLET and TOLLENS, being a modification of SCHWARZ'S [3] method:

Treat 12 liters 90-per cent alcohol with 480 cc. fuming hydrochloric acid and warm to 45–50° C.; gradually add 4 kilos of powdered cane-sugar, and allow to cool after two hours, when all the sugar will have dissolved and been inverted. To incite crystallization, some crystals of anhydrous dextrose are added, and after several days the crystals are sucked dry by the air-pump, washed with dilute alcohol to remove hydrochloric acid, and crystallized from alcohol or methyl alcohol. According to TOLLENS it is best to dissolve the sugar in one-half its weight of water on the water-bath and then add double this volume of 90–95-per cent alcohol.

In detecting dextrose in animal fluids or extracts of tissues we may make use of the above-mentioned reduction tests, the optical determination, fermentation, and phenylhydrazine tests. For the quantitative estimation the reader is referred to the chapter on the urine. Those liquids containing proteins must first have these removed by coagulation with heat and addition of acetic acid, or by precipitation with alcohol or metallic salts, before testing for dextrose. In regard to the difficulties of operating with blood and serous fluids we refer the student to larger works.

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[1] Ber. d. deutsch. chem. Gesellsch., **19**; also Kueny, Zeitschr. f. physiol. Chem., **14**, and Skraup, Wien. Sitzungsber., **98**, (1888).

[2] Molisch, Monatshefte f. Chem., **7**, and Centralbl. f. d. med. Wissensch., 1887, pp. 34 and 49; Reinbold, Pflüger's Arch., **103**.

[3] Tollens, Handbuch der Kohlehydrate, 2. Aufl. I, 39.

Mannoses. *d-Mannose,* also called *seminose,* is obtained with *d*-levulose on the careful oxidation of *d*-mannite. It is also obtained on the hydrolysis of natural carbohydrates, such as salep slime and reserve cellulose (especially from the shavings from the ivory-nut). It is dextrorotatory, readily ferments with beer-yeast, gives a hydrazone not readily soluble in water, and an osazone which is identical with that from *d*-glucose.

Galactose (not to be mistaken for lactose or milk-sugar) is obtained on the hydrolytic cleavage of milk-sugar and by hydrolysis of many other carbohydrates, especially varieties of gums and mucilaginous bodies. It is also obtained on heating cerebrin, a nitrogenized glucoside prepared from the brain, with dilute mineral acids.

It crystallizes in needles or leaves which melt at 168° C. It is somewhat less soluble in water than dextrose. It is dextrogyrate, and according to NEUBERG[1] has a rotation $(\alpha)_D = +81°$. With ordinary yeast galactose is slowly, but nevertheless completely, fermented. It is fermented by a great variety of yeasts (E. FISCHER and THIERFELDER), but not by Saccharomyces apiculatus,[2] which is of importance in physiological-chemical investigations. Galactose reduces FEHLING'S solution to a less extent than dextrose, and 10 cc. of this solution are reduced, according to SOXHLET, by 0.0511 gram galactose in 1-per cent solution. Its phenylosazone melts according to NEUBERG at 196–197° C., and is soluble with difficulty in hot water, but with relative ease in hot alcohol. Its solution in glacial acetic acid is optically inactive. In the test with hydrochloric acid and phloroglucin galactose gives a color similar to that of the pentoses, but the solution does not give the absorption spectrum. On oxidation it first yields galactonic acid and then mucic acid, and these serve in the detection of galactose.

Levulose, also called *d*-FRUCTOSE and FRUIT-SUGAR, occurs, as above stated, mixed with dextrose extensively distributed in the vegetable kingdom and also in honey. It is formed in the hydrolytic cleavage of cane-sugar and several other carbohydrates, but it is very readily obtained by the hydrolytic splitting of inulin. In extraordinary cases of diabetes mellitus we find levulose in the urine. NEUBERG and STRAUSS[3] have detected levulose with positiveness in human blood-serum, and exudates in certain cases.

Levulose crystallizes with comparative difficulty in coarse crusts or warts or in fine needles. C. MÖRNER[4] has obtained crystals 2–3 mm. long which belonged to the rhombic system, and neither melted nor lost in weight on heating to 100° C. The melting-point is 110° C. Levulose

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[1] See C. Oppenheimer, Handb. d. Biocehm. **1**, p. 197.

[2] See F. Voit, Zeitschr. f. Biol., **28** and **29**.

[3] Zeitschr. f. physiol. Chem., **36**, which also contains the older literature.

[4] Svensk. Farmac. Tidskr, No. 6, 1907. See also Maly's Jahresb., **37**, p. 95.

is readily soluble in water, but nearly insoluble in cold absolute alcohol, though rather readily in boiling alcohol. Its aqueous solution is levogyrate. C. MÖRNER found the rotation for a 10- and 20-per cent solution was $(\alpha)_D = -93°$ and $-94.1°$ respectively. Levulose ferments with yeast, and gives the same reduction tests as dextrose, and also the same osazone. It gives a compound with lime which is less soluble than the corresponding dextrose compound. Levulose is not precipitated by sugar of lead or basic lead acetate.

Levulose does not reduce copper to the same extent as dextrose. Under similar conditions the reduction relationship is 100: 92.08.

In detecting levulose and those varieties of sugar which yield levulose on cleavage we make use of the following reaction, suggested by SELIWANOFF: To a few cubic centimeters of fuming hydrochloric acid add an equal volume of water and a small quantity of the sugar solution or of the solid substance and a few crystals of resorcinol, and apply heat. The liquid becomes a beautiful red, and gradually a substance precipitates which is red in color and soluble in alcohol. According to OFNER[1] the mixture must not contain more than 12 per cent HCl, and the boiling must not be continued longer than twenty seconds, otherwise glucose, mannose, and indeed maltose, may give a similar reaction. R. and O. ADLER[2] perform the test with glacial acetic acid and a drop of hydrochloric acid and some resorcinol, in which case a reaction with aldoses is not obtained. SELIWANOFF'S reaction, which according to ROSIN may be made more delicate by a combination with the spectroscopic examination, is, as NEUBERG[3] has shown, a general reaction for ketoses.

The **naphtho-resorcinol** reaction as suggested by B. TOLLENS and RORIVE[4] can be carried out as follows: A few particles of the sugar and about the same quantity of naphthoresorcinol are treated with about 10 cc. of a mixture of equal volumes of water and concentrated hydrochloric acid. of sp. gr. 1.19. This is slowly heated to boiling over a low flame, and this continued for 1–3 minutes. The fluid becomes more purple or violet than with SELIWANOFF'S resorcin test. The spectroscopic examination shows a faint band in the green.

According to NEUBERG,[5] methylphenylhydrazine is an excellent substance to use for the separation and detection of levulose, as it gives a characteristic levulose methylphenylosazone. This osazone when recrystallized from alcohol melts at 153°. It shows a dextrorotation of 1° 40′

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[1] Monatshefte f. Chem., **25**.

[2] See foot-note 5, p. 203.

[3] Zeitschr. f. physiol. Chem., **31**; Rosin, *ibid.*, **38**.

[4] Ber. d. d. chem. Gesellsch., **41**, p. 1783 and Tollens, *ibid.*, **41**, p. 1788. See also Mandel and Neuberg, Biochem. Zeitschr. **13**.

[5] Ber. d. d. chem. Gesellsch., **35**; also Neuberg and Strauss, *ibid.*, **36**.

when 0.2 gram of the osazone is dissolved in 4 cc. pyridine and 6 cc. absolute alcohol.

OFNER has made objections to the use of methylphenylhydrazine in the detection of levulose. He has obtained the osazone from dextrose and methylphenlhydrazine, although the osazone is formed much more quickly with levulose than with dextrose. Only when the separation of the osazone crystals with methylphenylhydrazine after the addition of acetic acid takes place within five hours at ordinary temperatures is the presence of levulose positively proven (OFNER [1]).

The use of secondary asymmetric hydrazines as a general reagent for ketoses and as a means of separation from aldoses is objected to by OFNER.

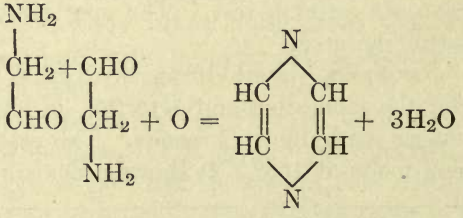
Levulose, as above stated, is best obtained by the hydrolytic cleavage of inulin, by warming with faintly acidulated water.

d-**Sorbinose** (sorbin) is a ketose obtained from the juice of the berry of the mountain ash under certain conditions. It is crystalline and levogyrate, and is converted into *d*-sorbite by reduction.

Appendix to the Monosaccharides.

a. Amino Sugars.

The amino sugars, as intermediary bodies between the carbohydrates and oxyamino-acids, are of great physiological interest, and this interest has become still more important since NEUBERG was first able to prepare the corresponding amino-aldehyde from glycocoll and then also from other amino-acids. From the ethyl ester of glycocoll in acid solution NEUBERG [2] obtained the amino-acetaldehyde, $NH_2.CH_2.CHO$, by treatment with sodium amalgam. This aldehyde is very unstable and has a tendency to condensation with ring formation, and NEUBERG obtained therefrom by oxidation with corrosive sublimate and caustic soda, pyrazine according to the equation:

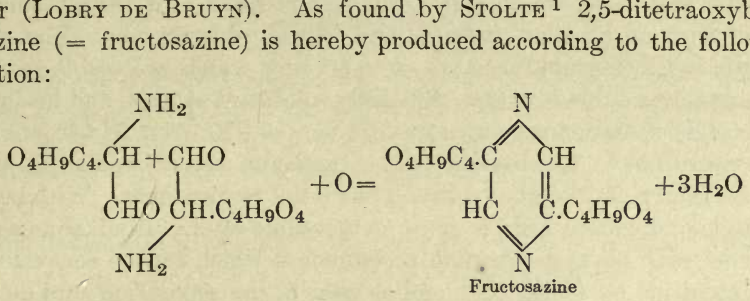


On account of this tendency to ring-formation the amino-acetaldehyde as well as the amino-aldehydes as a group, stand, according to NEU-

[1] Ber. d. d. chem. Gesellsch., **37**, and Zeitschr. f. physiol. Chem., **45**.
[2] *Ibid.*, **41**.

BERG, in close relationship to many ring systems, such as imidazole, piperazine, pyrazine, pyridine and others, and also to the alkaloids.

The amino-sugars, like the amino-aldehydes, can also unite, forming ring compounds, and this seems to be the case on the decomposition of free glucosamine in aqueous solution, which occurs with access of air (LOBRY DE BRUYN). As found by STOLTE [1] 2,5-ditetraoxybutyl pyrazine (= fructosazine) is hereby produced according to the following equation:



Fructosazine

The 2,5-ditetraoxybutyl pyrazine, which STOLTE obtained by LOBRY DE BRUYN'S [2] method from levulose in methyl alcohol solution and ammonia, and which he calls *fructosazine*, can be oxidized outside of the body into 2,5-pyrazine dicarboxylic acid.

The same acid can be formed in the animal body (rabbits), although not constantly, after introducing fructosazine. It also passes into the urine of rabbits after intravenous injection of *d*-levulose and glycocoll (SPIRO), a behavior which SPIRO claims indicates that carbohydrates in metabolism react with the cleavage products of proteins. STOLTE'S experiments to decide the question whether in the animal body the glucosamine in its decomposition passes into fructosazine did not at first yield conclusive results. His more recent investigations [3] show on the contrary that in rabbits 2-oxymethylpyrazine-5-carboxylic acid is formed as an oxidation product, and this can be oxidized outside of the body into pyrazine-2,5-dicarboxylic acid.

According to OFFER [4], pentosamine occurs in the liver of the horse. According to OFFER, the pentose derivative, which he calls dipentosamine $(C_5H_7O_3.NH_2)_2 + H_2O$ and a second, perhaps a diacetyl-pentosamine $2(CH_3CO)C_{10}H_{18}N_2O_7$ (?), also occur in the liver. The first gives pentose reactions and reduces FEHLING'S solution after boiling with acid. The only amino-sugar positively detected in the animal organs is glucosamine.

***d*-Glucosamine** [5] (chitosamine), $C_6H_{13}NO_5$, $= \begin{matrix} CH_2OH \\ (CH.OH)_3 \\ CH.NH_2 \\ COH \end{matrix}$, whose synthetical preparation has already been given on page 197, was first prepared

[1] Hofmeister's Beiträge, **11**.

[2] Cited by Stolte, Hofmeister's Beiträge, **11**.

[3] Spiro, Hofmeister's Beiträge, **10**, p. 283; Stolte, Biochem. Zeitschr., **12**.

[4] Hofmeister's Beiträge, 8.

[5] According to E. Fischer's suggestion we shall use the term glucosamine instead of the term chitosamine, which has lately been generally used.

by LEDDERHOSE [1] from chitin by the action of concentrated hydrochloric acid. Recently it has been obtained as a cleavage product of several mucin substances and proteins (see pages 83 and 164). Glucosamine is, as E. FISCHER and LEUCHS [2] have shown, a derivative of glucose or *d*-mannose (probably glucose), and is a α-amino-sugar.

The free base, which can crystallize in needles, is readily soluble in water with an alkaline reaction, and quickly decomposes. The characteristic hydrochloride forms colorless crystals which are stable in the air and readily soluble in water, difficultly soluble in alcohol, and insoluble in ether. The solution is dextrorotatory, $(\alpha)_D = +70.15°$ to $74.64°$, at various concentrations.[3] Glucosamine has a reducing action similar to that of glucose, and gives the same osazone, but is not fermentable. With benzoyl-chloride and caustic soda it gives a crystalline ester. In alkaline solution it gives with phenylisocyanate a compound which can be converted into its anhydride by acetic acid, and is used in the separation and detection of glucosamine (STEUDEL [4]). On oxidation with nitric acid it yields norisosaccharic acid, whose lead salt can be separated, and whose salts with cinchonine or quinine are difficultly soluble in water and can also be used very successfully in the detection of glucosamine (NEUBERG and WOLFF [5]). On oxidation with bromine, chitaminic acid (*d*-glucosaminic acid) is produced, and this is converted into chitaric acid, $C_6H_{10}O_6$, by nitrous acid. On treatment with nitrous acid glucosamine yields a non-fermentable sugar called chitose.

EHRLICH [6] has suggested a test which does not respond with the free glucosamine, but with the mucins and other protein bodies containing an acetylated glucosamine. It consists in warming the substance, which has previously been treated with alkali, with a hydrochloric-acid solution of dimethylaminobenzaldehyde, when a beautiful red color is obtained.

Glucosamine is best prepared from decalcified lobster-shells by treating with hot concentrated hydrochloric acid.[7] In regard to its preparation from protein substances we must refer to the works cited on page 83, footnote 3.

Albamine (diglucosamine), $(C_6H_{11}O_4N)_2 + H_2O$, is the name given by S. FRÄNKEL [8] to a body which he isolated from the products of the hydrolysis of ovalbumin with baryta, as well as in its digestion. Albamine is amorphous, dextrogyrate,

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[1] Zeitschr. f. physiol. Chem., **2** and **4**.

[2] Ber. d. d. chem. Gesellsch., **36**.

[3] See Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.; Sundwik, Zeitschr. f. physiol. Chem., **34**.

[4] Zeitschr. f. physiol. Chem., **34**.

[5] Ber. d. d. chem. Gesellsch., **34**.

[6] Mediz. Woche, 1901, No. 15; see Langstein, Ergebnisse der Physiol., I, Abt. 1, 88.

[7] See Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

[8] Monatsh. f. Chem., **19**.

and reduces after boiling with acids. As hydrolytic cleavage product it yields *d*-glucosamine.

Galactosamine is claimed to have been found by SCHULZ and DITTHORN in a glycoprotein of the spawn of the frog. This claim is not generally accepted. v. EKENSTEIN and BLANKSMA [1] obtained galactose on the hydrolysis of the slimy envelope of frog eggs.

b. Glucuronic Acids.

The glucuronic acids occurring in the animal body either physiologically or pathologically, are conjugated acids which will be described in detail in a subsequent chapter (XV). We here will only describe the *d*-glucuronic acid in connection with the carbohydrates.

***d*-Glucuronic acid** (glycuronic acid), $C_6H_{10}O_7 = \begin{matrix}CHO\\(CH.OH)_4\\COOH\end{matrix}$, is a derivative of dextrose, and has been synthetically prepared by E. FISCHER and PILOTY [2] by the reduction of the lactone of saccharic acid. On oxidation with bromine it forms saccharic acid, and on reduction it yields gulonic-acid lactone. SALKOWSKI and NEUBERG [3] have obtained *l*-xylose from glucuronic acid by splitting off CO_2 by means of putrefaction bacteria.

Glucuronic acid has not been found in the free state in the animal body. It occurs to a slight extent in normal urine as a conjugated acid, phenol and probably also indoxyl- and skatoxylglucuronic acid (MAYER and NEUBERG). It occurs to a much greater extent in urine as conjugated acid after the ingestion of certain aromatic and also aliphatic substances, especially camphor and chloral hydrate. It was obtained first by SCHMIEDEBERG and MEYER from camphoglucuronic acid, and then by v. MERING [4] from urochloralic acid by cleavage with dilute acids. According to P. MAYER,[5] on the oxidation of dextrose a partial formation of glucuronic acid and oxalic acid takes place, and therefore, according to him, an increased elimination of conjugated glucuronic acids shows in certain cases an incomplete oxidation of dextrose. Conjugated glucuronic acids may also occur in the blood (P. MAYER, LÉPINE and BOULUD [6]), in the feces, and in the bile.[7] NEUBERG and NEIMANN [8] have prepared

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[1] Schulz and Ditthorn, Zeitschr. f. physiol. Chem., **29**; v. Ekenstein and Blanksma, Chem. Centralbl., 1907, **2**, p. 1001.

[2] Ber. d. d. chem. Gesellsch., **24**.

[3] Zeitschr. f. physiol. Chem., **36**.

[4] Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Schmiedeberg u. Meyer, *ibid.*, **3**; v. Mering, *ibid.*, **6**.

[5] Zeitschr. f. klin. Med., **47**. See Chapter XV.

[6] Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. rend., **133**, **134**, **138**.

[7] See Bial, Hofmeister's Beiträge, **2**, and v. Leersum, *ibid.*, **3**.

[8] Zeitschr. f. physiol. Chem., **44**.

certain conjugated glucuronic acids (see Chapter XV) synthetically, among them being euxanthic acid. The most abundant source of glucuronic acid is the artist's pigment "Jaune indien," which contains the magnesium salt of euxanthic acid (euxanthon-glucuronic acid).

Glucuronic acid is not crystalline, but is only obtainable as a syrup. It dissolves in alcohol and is readily soluble in water. If the aqueous solution is boiled for an hour the acid is partly (20 per cent) converted into the crystalline lactone, glucurone, $C_6H_8O_6$, which is soluble in water and insoluble in alcohol, and which has a melting-point of 175–178° C.° The alkali salts of the acid are crystalline. If a concentrated solution of the acid is saturated with barium hydroxide the basic barium salt is obtained as a precipitate. The neutral lead salt is soluble in water, while the basic salt is insoluble. The readily crystallizable cinchonine salt can be used in isolating glucuronic acid (NEUBERG [1]). Glucuronic acid is dextrorotatory, while the conjugated acids are levorotatory; they behave like dextrose with the reduction tests, and do not ferment with yeast. They give the pentose reactions with phloroglucin or orcin and hydrochloric acid, and also a good reaction with naphthoresorcinol and hydrochloric acid (see page 211). The product produced herewith is soluble in ether with a blue, bluish-violet or reddish-violet color, and the solution shows an absorption band somewhat to the right and on the *D*-line. According to MANDEL and NEUBERG [2] this reaction is not characteristic of glucuronic acid, as many aldehyde and ketone acids give the same reaction; still, it is important in the differentiation of the pentoses. With the phenylhydrazine test it gives crystalline compounds which are not sufficiently characteristic (THIERFELDER, P. MAYER [3]). By the action of 3 mol. phenylhydrazine and the necessary amount of acetic acid upon 1 mol. glucuronic acid at 40° for a few days, NEUBERG and NEIMANN obtained the glucuronic-acid osazone, which was very similar to glucosazone and melted at 200–205°. With *p*-bromphenylhydrazine hydrochloride and sodium acetate, glucuronic acid gives *p*-bromphenylhydrazine glucuronate, which is characterized by its insolubility in absolute alcohol and by a very prominent levorotatory action. This compound is very well suited for the detection of glucuronic acid.[4] Dissolved in a mixture of alcohol and pyridine (0.2 gram substance in 4 cc. pyridine and 6 cc. alcohol) the rotation is 7° 25′, which corresponds to $(\alpha)\frac{20}{D} = -369°$. On distillation with hydrochloric acid, glucuronic acid yields furfurol and also carbon dioxide, and on this behavior TOLLENS

[1] Ber. d. d. chem. Gesellsch., **33**.

[2] Biochem. Zeitschr., **13**.

[3] Thierfelder, Zeitschr. f. physiol. Chem., **11**, **13**, **15**; P. Mayer, *ibid.*, **29**.

[4] See Neuberg, Ber. d. d. chem. Gesellsch., **32**; and Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

and LEFÈVRE [1] have based their quantitative method for the estimation of glucuronic acid.

Glucuronic acid is best prepared from euxanthic acid, which decomposes on heating it with water to 120° C. for several hours. The filtrate from the euxanthon is concentrated at 40° C., when the anhydride gradually crystallizes out. On boiling the mother-liquor for some time and evaporating further, the crystals of the lactone are obtained. In regard to the quantitative estimation of glucuronic acid we must refer the reader to the works of TOLLENS and his collaborators and of NEUBERG and NEIMANN.[2]

2. Disaccharides.

Some of the varieties of sugar belonging to this group occur ready formed in nature. Thus we have saccharose and lactose. Some, on the contrary, such as maltose and isomaltose, are produced by the partial hydrolytic cleavage of complex carbohydrates. Isomaltose is also obtained from dextrose by reversion (see page 219).

The disaccharides or hexobioses are to be considered as glucosides, each of which is derived from two monosaccharides with the exit of 1 molecule of water. Corresponding to this, their general formula is $C_{12}H_{22}O_{11}$. On hydrolytic cleavage and the addition of water they yield 2 molecules of hexoses, either 2 molecules of the same hexose or one each of two different hexoses. Thus

$$\text{Saccharose} + H_2O = \text{dextrose} + \text{levulose};$$
$$\text{Maltose} + H_2O = \text{dextrose} + \text{dextrose};$$
$$\text{Lactose} + H_2O = \text{dextrose} + \text{galactose}.$$

The configuration of the dissaccharides has not been determined with positiveness.

The levulose turns the polarized ray more to the left than the dextrose does to the right; hence the mixture of hexoses obtained on the cleavage of saccharose has an opposite rotation to the saccharose itself. On this account the mixture is called INVERT-SUGAR, and the hydrolytic splitting is designated as *inversion*. This term, "inversion," is not only used for the splitting of saccharose, but is also used for the hydrolytic cleavage of compound sugars into monosaccharides. The reverse reaction, whereby monosaccharides are condensed into complex carbohydrates, is called *reversion*.

We subdivide the disaccharides into two groups, first, the group to which saccharose belongs, where the members do not have the property

[1] Ber. d. d. chem. Gesellsch., **40**.

[2] Tollens, Zeitschr. f. physiol. Chem., **44**, which cites also the older work; Neuberg and Neimann, *ibid.*, **44**; Neuberg, *ibid.*, **45**.

of reducing certain metallic oxides; and the second group, to which the two maltoses and lactose belong, the members acting like monosaccharides in regard to the ordinary reduction tests. The members of the latter group have the character of aldehyde alcohols, and in milk-sugar the aldehyde characteristics are connected with the glucose fraction.

Saccharose, or CANE-SUGAR, occurs extensively distributed in the plant kingdom. It occurs to the greatest extent in the stalk of the sugar-millet and sugar-cane, the roots of the sugar-beet, the trunks of certain varieties of palms and maples, in carrots, etc. Cane-sugar is of extraordinarily great importance as a food and condiment.

Saccharose forms large, colorless monoclinic crystals. On heating it melts in the neighborhood of 160° C., and on heating more strongly it turns brown, forming so-called caramel. It dissolves very readily in water, and according to SCHEIBLER,[1] 100 parts of saturated saccharose solution contain 67 parts of sugar at 20° C. It dissolves with difficulty in strong alcohol. Cane-sugar is strongly dextrorotatory. The specific rotation is only slightly modified by concentration, but is markedly changed by the presence of other inactive substances. The specific rotation is $(\alpha)_D = +66.5°$.

Saccharose acts indifferently toward MOORE's test and to the ordinary reduction tests. On continuous boiling it may reduce an alkaline copper solution, perhaps on account of a partial hydrolysis. It does not ferment directly, but only after inversion, which can be brought about by an enzyme (invertin) contained in the yeast. An inversion of cane-sugar also takes place in the intestinal canal. Cane-sugar does not combine with hydrazines. Concentrated sulphuric acid blackens cane-sugar very quickly even at the ordinary temperature, and anhydrous oxalic acid does the same on warming on the water-bath. Various products are obtained on the oxidation of cane-sugar, dependent upon the variety of oxidizing agent and also upon the intensity of the action. Saccharic acid and oxalic acid are the most important products.

The reader is referred to complete text-books on chemistry for the preparation and quantitative estimation of cane-sugar.

Maltose (MALT-SUGAR) is formed in the hydrolytic cleavage of starch by malt diastase, saliva, and pancreatic juice. It is obtained from glycogen under the same conditions (see Chapter VIII). Maltose is also produced transitorily in the action of sulphuric acid on starch. Maltose forms the fermentable sugar of the potato or grain mash, and also of the beerwort.

Maltose crystallizes with one molecule water of crystallization in fine white needles. It is readily soluble in water, rather easily in alcohol,

[1] See Tollen's Handbuch der Kohlehydrate, 2. Aufl. **1**, 124.

but insoluble in ether. Its solutions are dextrorotatory; and the specific rotation is variable, depending upon the concentration and temperature, but is considerably stronger than dextrose,[1] and is generally given as $(\alpha)_D = +137$ to 138°. Maltose ferments readily and completely with yeast, and acts like dextrose in regard to the reduction tests. It yields phenylmaltosazone on warming with phenylhydrazine for 1½ hours. This phenylmaltosazone melts at 205° C., and is more soluble in hot water than the glucosazone. Maltose differs from dextrose chiefly in the following: It does not dissolve as readily in alcohol, has a stronger dextrorotatory power, and has a feebler reducing action on FEHLING'S solution; 10 cc. FEHLING'S solution are, according to SOXHLET,[2] reduced by 77.8 milligrams anhydrous maltose in approximately 1-per cent solution.

Isomaltose. This variety of sugar, as has been shown by FISCHER,[3] is produced, as are dextrin-like products, by reversion, and by the action of fuming hydrochloric acid on dextrose. A re-formation of isomaltose and other sugars from dextrose can also be brought about by means of yeast maltase (HILL and EMMERLING[4]). It is also formed, besides ordinary maltose, in the action of diastase on starch paste, and occurs in beer and in commercial starch-sugar. The formation of isomaltose in the hydrolysis of starch by malt diastase has been denied by many investigators because they considered isomaltose as contaminated maltose.[5] It is produced, with maltose, by the action of saliva or pancreatic juice (KÜLZ and VOGEL) or blood-serum (RÖHMANN[6]) on starch.

Isomaltose dissolves very readily in water, has a pronounced sweetish taste, and does not ferment, or, according to some, only very slowly. It is dextrorotatory, and has very nearly the same power of rotation as maltose. Isomaltose is characterized by its osazone. This forms fine yellow needles, which begin to form drops at 140° C. and melt at 150–153° C. These are rather easily soluble in hot water and dissolve in hot absolute alcohol much more readily than the maltosazone. Isomaltose reduces copper as well as bismuth solutions.

Lactose (MILK-SUGAR). As this sugar occurs exclusively in the animal world, in the milk of human beings and animals, it will be treated in a following chapter (on milk).

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[1] See Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

[2] Cited from Tollens' Handbuch der Kohlehydrate, 2. Aufl. 1, 154.

[3] Ber. d. deutsch. chem. Gesellsch., **23** and **28**.

[4] Emmerling, *ibid.*, **34**; Hill, *ibid.*, **34**, and l. c., foot-notes 1 and 2, p. 65.

[5] Brown and Morris, Journ. of Chem. Soc., 1895; Chem. News, **72**. See also Ost. Ulrich, and Jalowetz, Ref. in Ber. d. deutsch. chem. Gesellsch., **28**; Ling and Baker, Journ. of Chem. Soc., 1895; Pottevin, Chem. Centralbl., 1899, II, 1023.

[6] Külz and Vogel, Zeitschr. f. Biologie, **31**; Röhmann, Centralbl. f. d. med. Wissensch., 1893, 849.

3. Colloid Polysaccharides.

If we exclude the not well known trisaccharides and the tetrasaccharide *stachyose*, this group includes a great number of very complex carbohydrates which occur only in the amorphous condition, or at least not as crystals in the ordinary sense. Unlike the bodies belonging to the other groups, these have no sweet taste. Some are soluble in water, while others swell up therein, especially in warm water, and finally some are neither dissolved nor visibly changed. Polysaccharides are ultimately converted into monosaccharides by hydrolytic cleavage.

The polysaccharides are ordinarily divided into the following groups: *starches* with the *dextrins*, *plant gums* and *mucilages*, and the *celluloses*.

Starch Group.

Starch, AMYLUM $(C_6H_{10}O_5)x$. This substance occurs in the plant kingdom very extensively distributed in the different parts of the plant, especially as reserve food in the seed, roots, tubers, and trunks.

Starch is a white, odorless, and tasteless powder, consisting of small granules which have a stratified structure and different shape and size in different plants. Starch is considered insoluble in cold water. The grains swell up in warm water and burst, yielding a paste.

According to the ordinary opinion the starch granules consist of two different substances, STARCH GRANULOSE and STARCH CELLULOSE (v. NÄGELI), the first of which turns blue with iodine and forms the chief part of the granule. According to MAQUENNE and ROUX[1] this is not the fact. According to them the starch granule consists of two constituents, of which the first, *amylose*, forms the chief mass (80–85 per cent) and the other, *amylopectin*, forms only 15–20 per cent of the granule. Amylopectin is not identical with v. NÄGELI's starch cellulose, and the above investigators consider starch cellulose as only an insoluble form of amylose. The amylose can occur in two forms. One, which is soluble, is colored blue by iodine and is immediately transformed into sugar by malt. The other is a solid substance, which is not colored with iodine and resists the action of malt infusion. One modification can be transformed into the other.

In the paste, besides amylopectin, we also have soluble amylose, and this can, by a process called *retrogradation* by MAQUENNE and ROUX, be transformed into the solid modification, into "artificial starch." This solid form occurs in the starch granule, and is identical with v. NÄGELI's starch cellulose. As the starch granules are directly colored blue by

[1] v. Nägeli, Botan. Mitteil., 1863; Maquenne and Roux, Compt. rend., **138**, **140**, **142**, **146**, and Bull. Soc. chim. de Paris (3), **33** and **35**.

iodine they must, besides this, also contain soluble amylose. If the author understands the above investigators correctly the starch granules contain three constituents, namely; soluble amylose, which is colored blue by iodine (=starch granulose), insoluble amylose, which is not colored by iodine (=starch cellulose), and amylopectin.

In the formation of paste the amount of amylose is not the essential, but rather the quantity of amylopectin. The amylopectin is a slime-like substance, insoluble in boiling water and dilute alkalies, only becoming pasty therein, and not colored blue by iodine. Accordingly the paste is a solution of amylose made thick by amylopectin. The amylopectin, unlike the amylose, is only slowly transformed into sugar with dextrin formation. Starch is insoluble in alcohol and ether. On heating starch with water alone, or heating with glycerin to 190° C., or on treating the starch grains with 6 parts dilute hydrochloric acid of sp. gr. 1.06 at ordinary temperature for six to eight weeks,[1] it is converted into soluble starch (AMYLODEXTRIN, AMIDULIN). Soluble starch is also formed as an intermediate step in the conversion of starch into sugar by dilute acids or diastatic enzymes. Soluble starch may be precipitated from very dilute solutions by baryta-water.[2]

Starch granules swell up and form a pasty mass in caustic potash or soda. This mass gives neither MOORE'S nor TROMMER'S test. Starch paste does not ferment with yeast. The most characteristic test for starch is the blue coloration produced by iodine in the presence of hydriodic acid or alkali iodides.[3] This blue coloration disappears on the addition of alcohol or alkalies, and also on warming, but reappears again on cooling.

On boiling with dilute acids starch is converted into dextrose. In the conversion by means of diastatic enzymes we have, as a rule, besides dextrin, maltose, and isomaltose, only very little dextrose. We are considerably in the dark as to the kind and number of intermediate products produced in this process (see Dextrins).

Starch may be detected by means of the microscope and by the iodine reaction. Starch is quantitatively estimated, according to SACHSSE'S method,[4] by converting it into dextrose by hydrochloric acid and then determining the dextrose by the ordinary methods.

Inulin, $(C_6H_{10}O_5)x+H_2O$, occurs in the underground parts of many Compositæ, especially in the roots of the Inula helenium, the tubers

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[1] See Tollens' Handb., 191. In regard to other methods, see Wróblewsky, Ber. d. deutsch. chem. Gesellsch., **30**; Syniewski, *ibid.*

[2] In regard to the compounds of soluble starch and dextrins with barium hydroxide, see Bülow, Pfluger's Arch., **62**.

[3] See Mylius, Ber. d. deutsch. chem. Gesellsch., **20**, and Zeitsch. f. physiol. Chem., **11**.

[4] Tollen's Handb., 2. Aufl., **1**, 187.

of the Dahlia, the varieties of Helianthus, etc. It is ordinarily obtained from the tubers of the Dahlia.

Inulin forms a white powder similar to starch, consisting of spheroid crystals which are readily soluble in warm water without forming a paste. It separates slowly on cooling, but more rapidly on freezing. Its solutions are levogyrate and are precipitated by alcohol, and are colored only yellow with iodine. Inulin is converted into the levogyrate monosaccharide *d*-levulose on boiling with dilute sulphuric acid. Diastatic enzymes have no, or only a very slight, action on inulin.[1]

According to DEAN [2] inulin occurs in combination with other substances, *levulins*, which are more soluble and have less rotation. He suggests that we limit the name inulin to that carbohydrate (or mixture of carbohydrates), which is readily precipitable by 60-per cent alcohol and shows a specific rotation of $(\alpha)_D = -38–40°$.

Lichenin (MOSS-STARCH) occurs in many lichens, especially in Iceland moss. It is not soluble in cold water, but swells up into a jelly. It is soluble in hot water, forming a jelly on allowing the concentrated solution to cool. It is colored yellow by iodine and yields glucose on boiling with dilute acids. Lichenin is not changed by diastatic enzymes such as ptyalin or amylopsin (NILSON [3]).

Glycogen. This carbohydrate, which stands to a certain extent between starch and dextrin, is principally found in the animal kingdom, hence it will be considered in a subsequent chapter (on the liver).

Dextrins and Gums.

The dextrins stand in close relation to the starches, and are formed therefrom as intermediate products by the action of acids or diastatic enzymes. They yield as final products only hexoses, indeed only dextrose, on complete hydrolysis. The vegetable gums, the vegetable mucilages and the pectin bodies, which all stand close to the hemicelluloses, yield, on the contrary, abundance of pentose and, among the hexoses, a galactose is very often found.

Dextrin (starch-gum, British gum) is produced on heating starch to 200–210° C., or by heating starch, which has previously been moistened with water containing a little nitric acid, to 100–110° C. Dextrins are also produced by the action of dilute acids and diastatic enzymes on starch. There have been numerous investigations as to the steps involved in the last-mentioned process, but they have led to conflicting views. One of these, which used to be generally accepted, is as follows: The first product, which gives a blue color with iodine, is soluble starch or *amylodextrin*, which on further hydrolytic cleavage yields sugar and *erythrodextrin*, which is colored red by iodine. On further cleavage of this erythrodextrin more sugar and a dextrin, *achroodextrin*, which is not colored by iodine, is formed. From this achroodextrin after successive splittings we have sugar and dextrins of lower molecular weights

[1] Tollen's Handbuch, 208. [2] Amer. Chem. Journ., **32**. [3] Upsala Läkaref. Förh., 28.

formed, until finally we have sugar and a dextrin, *maltodextrin*, which refuses to split further, as final products. The views are rather contradictory in regard to the number of dextrins which occur as intermediate steps. The sugar formed is maltose (or in first place isomaltose), and only very little dextrose is produced. Another view is that first several dextrins are formed consecutively in the successive splittings, by hydration, and then finally the sugar is formed by the splitting of the last dextrin. According to MOREAU, in the first stages of saccharification amylodextrin, erythrodextrin, achroodextrin and sugar are formed simultaneously. Other investigators, especially SYNIEWSKI, have recently suggested other views on the subject.[1]

This question has taken another direction by the investigations of MAQUENNE, mentioned above. According to him the amylose passes directly into maltose without the formation of dextrin by the action of malt infusion. The dextrins produced are only formed from the amylopectin, which does not undergo saccharification with freshly prepared malt infusions, but only with older or especially active infusions. This also explains why in the older investigations the saccharification was only about 80 per cent while MAQUENNE has been able to completely convert the starch into sugar by enzymotic action.

The various dextrins are very hard to isolate as chemical individuals and to separate from each other. YOUNG[2] has tried their separation by means of neutral salts, especially ammonium sulphate, and MOREAU by the aid of a baryta-alcohol method. We cannot enter into the differences as to the dextrins so separated, and only the characteristic properties and reactions will be given for the dextrins in general.

The dextrins appear as amorphous, white or yellowish-white powders which are readily soluble in water. Their concentrated solutions are viscid and sticky, like gum solutions. The dextrins are dextrogyrate. They are insoluble or nearly so in alcohol, and insoluble in ether. Watery solutions of dextrins are not precipitated by basic lead acetate. Dextrins dissolve cupric hydroxide in alkaline liquids, forming a beautiful blue solution, which, as is generally admitted, is reduced by pure dextrins. According to MOREAU pure dextrin has no reducing action. The dextrins are not directly fermentable.

The **vegetable gums** are soluble in water, forming solutions which are viscid but may be filtered. We designate, on the contrary, as **vegetable mucilages**

[1] In regard to the various views on the theories of the saccharification of starch, see Musculus and Gruber, Zeitschr. f. physiol. Chem., 2; Lintner and Düll, Ber. d. d. chem. Gesellsch., 26 and 28; Brown and Heron, Journ. of Chem. Soc., 1879; Brown and Morris, *ibid.*, 1885 and 1889; Moreau, Biochem. Centralbl., 3, 648; Syniewski, Annal. d. Chem. u. Pharm., 309, and Chem. Centralbl., 1902, 2.

[2] Journ. of Physiol., 22, which contains the older researches of Nasse, Krüger, Neumeister, Pohl, and Halliburton. Moreau, l. c.

those varieties of gum which do not or only partly dissolve in water, and which swell up therein to a greater or less extent. The natural varieties of gum and mucilage, to which belong several generally known and important substances, such as gum arabic, wood-gum, cherry-gum, salep, and quince mucilage, and probably also the little-studied pectin substances, will not be treated in detail, because of their unimportance from a physiological standpoint.

The Cellulose Group $(C_6H_{10}O_5)x$.

Cellulose is that carbohydrate, or perhaps more correctly, mixture of carbohydrates, which forms the chief constituent of the walls of the plant-cells. This is true for at least the walls of the young cells, while in the walls of the older cells the cellulose is extensively incrusted with a substance called LIGNIN, and with many other cellulose derivatives and compounds.

The true celluloses are characterized by their great insolubility. They are insoluble in cold or hot water, alcohol, ether, dilute acids, and alkalies. We have only one specific solvent for cellulose, and that is an ammoniacal solution of copper oxide called SCHWEITZER'S reagent. The cellulose may be precipitated from this solvent by the addition of acids, and obtained as an amorphous powder after washing with water.

Cellulose is converted into a substance, so-called AMYLOID, which gives a blue coloration with iodine, by the action of concentrated sulphuric acid. With oxidizing agents (nitric acid, etc.) oxycelluloses are produced. By the action of strong nitric acid or a mixture of nitric acid and concentrated sulphuric acid, celluloses are converted into nitric-acid esters or nitrocelluloses, which are highly explosive and have found great practical use.

The ordinary celluloses when treated at the ordinary temperature with strong sulphuric acid and then boiled for some time after diluting with water are converted into dextrose. In this case it must be observed, according to MAQUENNE, that it is not maltose that is produced as an intermediate step, but another disaccharide, called *cellose* or *cellobiose*.

Hemicelluloses are, according to E. SCHULZE,[1] those constituents of the cell-wall related to cellulose which differ from the ordinary cellulose by dissolving on heating with strongly diluted mineral acids, such as 1.25-per cent sulphuric acid, and of yielding arabinose, xylose, galactose, and mannose instead of dextrose. Those hemicelluloses which serve partly as reserve food and partly as support-substance, are very widely distributed in the plant kingdom.

The cellulose, at least in part, undergoes decomposition in the intestinal tract of man and animals. A closer discussion of the nutritive value of cellulose will be given in a future chapter (on digestion). The great importance of the carbohydrates in the animal economy and to animal metabolism will also be given in the following chapters.

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[1] E. Schulze, Zeitschr. f. physiol. Chem., **16** and **19**, with Castro, *ibid.*, **36**.

CHAPTER V.

ANIMAL FATS AND PHOSPHATIDES.

1. Neutral Fats and Fatty Acids.

THE fats form the third chief group of the organic food of man and animals. They occur very widely distributed in the animal and plant kingdoms. Fat occurs in all organs and tissues of the animal organism, though the quantity may be so variable that a tabular exhibit of the amount of fat in different organs is of little interest. The marrow contains the largest quantity, having over 96 per cent. The three most important deposits of fat in the animal organism are the intermuscular connective tissue, the fatty tissue in the abdominal cavity, and the subcutaneous connective tissues. In plants, the seeds and fruit and in certain instances also the roots, are rich in fat. Fat also occurs deposited, during the winter's rest, in the trunks of trees.

The fats consist almost entirely of so-called neutral fats, with only very small quantities of fatty acids. The neutral fats are esters of the triatomic alcohol, glycerin, with monobasic fatty acids. These esters are triglycerides; that is, the hydrogen atoms of the three hydroxyl groups of the glycerin are replaced by the fatty-acid radicals, and their general formula is therefore $C_3H_5.O_3.R_3$. The animal fats consist chiefly of esters of the three fatty acids, stearic, palmitic, and oleic acids. In certain fats, especially in milk-fat, glycerides of fatty acids such as butyric, caproic, caprylic, and capric acids also occur in considerable amounts. Besides the above-mentioned ordinary fatty acids, stearic, palmitic, and oleic acids, we also find in human and animal fat, exclusive of certain fatty acids only little studied, the following non-volatile fatty acids, as glycerides, namely, lauric acid, $C_{12}H_{24}O_2$, myristic acid, $C_{14}H_{28}O_2$, and arachidic acid, $C_{20}H_{40}O_2$. Of the unsaturated fatty acids, besides oleic acid, we probably also have in small quantities glycerides of acids of the linolic acid series $C_nH_{2n-4}O_2$ and of the linolenic acid series, $C_nH_{2n-6}.O_2$. In this case the question can be raised whether or not these acids are not derived from the phosphatides mixed with the fats. In the plant kingdom triglycerides of other fatty acids, such as lauric acid, myristic acid, linoleic acid, erucic acid, etc., sometimes occur abundantly. Besides these, oxyacids and high molecular alcohols

have been found in many plant fats. The extent to which traces of these oxyacids occur in the animal kingdom has not been thoroughly investigated, but the occurrence of monoxystearic acid seems to have been proven.[1] The occurrence of high molecular alcohols, although ordinarily only in small amounts, has on the contrary been positively shown in animal fat.

The animal fats are of the greatest interest and consist of a mixture of varying quantities of TRISTEARIN, TRIPALMITIN, and TRIOLEIN, having an average elementary composition of C 76.5, H 12.0, and O 11.5 per cent. It must be remarked that in animal fat (mutton and beef tallow) as well as in plant fat (olive-oil) mixed triglycerides, such as dipalmityl-olein distearyl-palmitin and distearyl-olein, occur, and that these mixed glycerides may also be prepared synthetically.[2]

Fats from different species of animals, and even from different parts of the same animal, have an essentially different consistency, depending upon the relative amounts of the different individual fats present. In solid fats—as tallow—tristearin and tripalmitin are in excess, while the less solid fats are characterized by a greater abundance of triolein. This last-mentioned fat is found in greater quantities proportionally in cold-blooded animals, and this accounts for the fact that the fat of these animals remains fluid at temperatures at which the fat of warm-blooded animals solidifies. Human fat from different organs and tissues contains, in full numbers, 67–85 per cent triolein.[3] The melting-point of different fats depends upon the composition of the mixtures, and it not only varies for fat from different tissues of the same animal, but also for the fat from the same tissues in various kinds of animals.[4]

Neutral fats are colorless or yellowish, and, when perfectly pure, odorless and tasteless. They are lighter than water, on which they float when in a molten condition. They are insoluble in water, dissolve in boiling alcohol, but separate on cooling—often in crystals. They are easily soluble in ether, benzene, chloroform, carbon disulphide and petroleum ether. The fluid neutral fats give an emulsion when shaken with a solution of gum or albumin. With water alone they give an emulsion only after vigorous and prolonged shaking, but the emulsion is not per-

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[1] Erben, Zeitschr. f. physiol. Chem., **30**; Bernert, Arch. f. exp. Path. u. Pharm., **49**.

[2] Guth, Zeitschr. f. Biologie, **44**; W. Hansen, Arch. f. Hygiene, **42**; Holde and Stange, Ber. d. d. chem. Gesellsch., **34**; Kreis and Hafner, *ibid.*, **36**.

[3] See Knöpfelmacher, " Untersuch. über das Fett im Säuglingsalter," etc., Jahrbuch f. Kinderheilkunde (N. F.), **45**, which also contains the older literature; Jaeckle, Zeitschr. f. physiol. Chem., **36**.

[4] According to Gilkin (Ber. d. d. chem. Gesellsch., **41**) the fat from bone-marrow and also other fats of animal and plant origin contain iron, which cannot be removed by water containing hydrochloric acid.

sistent. The presence of some soap causes a very fine and permanent emulsion to form easily. Fat produces spots on paper which do not disappear; it is not volatile; it boils at about 300° C. with partial decomposition, and burns with a luminous and smoky flame. The fatty acids have most of the above-mentioned properties in common with the neutral fats, but differ from them in being soluble in alcohol-ether, in having an acid reaction, and by not giving the acrolein test. The neutral fats generate a strong irritating vapor of acrolein, due to the decomposition of glycerin, $C_3H_5(OH)_3 - 2H_2O = C_3H_3.CHO$, when heated alone, or more easily when heated with potassium bisulphate or with other dehydrating substances.

The neutral fats may be split by the addition of the constituents of water according to the following equation:

$$C_3H_5(OR)_3 + 3H_2O = C_3H_5(OH)_3 + 3HOR.$$

This splitting may be produced by the pancreatic enzyme and other enzymes occurring in the animal and vegetable kingdoms, for example, the castor lipase. As shown by Pottevin and Dietz[1] the reverse action, namely, the synthesis of fatty acid esters, can be brought about by enzymes, such as pancreatic lipase. The cleavage of the neutral fats can also be accomplished by superheated steam or by dilute acids. We most frequently decompose the neutral fats by boiling them with not too concentrated caustic alkali, or, still better (in biochemical researches), with an alcoholic potash solution or with sodium alcoholate. By this procedure, which is called saponification, the alkali salts of the fatty acids (soaps) are formed. If the saponification is made with lead oxide, then lead plaster, the lead salt of the fatty acids, is produced. By saponification is to be understood not only the cleavage of neutral fats by alkalies, but also the splitting of neutral fats into fatty acids and glycerin in general.

On keeping fats for a long time in contact with air they undergo a change, becoming yellow in color and acid in reaction, and they develop an unpleasant odor and taste, becoming *rancid*. In this change a part of the fat is split into fatty acids and glycerin, and then an oxidation of the free fatty acids takes place, producing volatile bodies of an unpleasant odor.

The three most important fats of the animal kingdom are *stearin*, *palmitin*, and *olein*.

Stearin, or tristearin, $C_{57}H_{110}O_6, = \begin{matrix} CH_2.O.C_{18}H_{35}O \\ CH.O.C_{18}H_{35}O \\ CH_2.O.C_{18}H_{35}O \end{matrix}$, occurs especially in

[1] Pottevin, Compt. rend., **138**, and Bull. soc. chim. (3), **35**; Dietz, Zeitschr. f. physiol. Chem., **52**.

the solid varieties of tallow but also in the vegetable fats. Stearic acid, $C_{18}H_{36}O_2$, is found in the free state in decomposed pus, in the expectorations in gangrene of the lungs, and in cheesy tuberculous masses. It occurs as lime soap in excrement and adipocere, and in this last product also as an ammonium soap. It also exists as alkali soap in the blood, bile, transudations and pus, and in the urine to a slight extent.

Stearin is the hardest and most insoluble of the three ordinary neutral fats. It is nearly insoluble in cold alcohol, and soluble with great difficulty in cold ether (225 parts). It separates from warm alcohol on cooling as rectangular, less frequently as rhombic plates. The opinions regarding the melting-point are somewhat varied. Pure stearin, according to HEINTZ,[1] melts transitorily at 55° and permanently at 71.5°. The stearin from the fatty tissues (not pure) melts at 63° C.

Stearic acid, $\begin{matrix}CH_3\\(CH_2)_{16}\\COOH\end{matrix}$, crystallizes (on cooling from boiling alcohol) in large, shining, long rhombic scales or plates. It is less soluble than the other fatty acids and melts at 69.2° C. Its barium salt contains 19.49 per cent barium, and its silver salt contains 27.59 per cent silver.

Palmitin, or tripalmitin, $C_{51}H_{98}O_6, = \begin{matrix}CH_2.O.C_{16}H_{31}O\\CH.O.C_{16}H_{31}O\\CH_2.O.C_{16}H_{31}O\end{matrix}$. Of the two solid varieties of fats, palmitin is the one which occurs in predominant quantities in human fat (LANGER[2]). Palmitin is present in all animal fats and in several kinds of vegetable fat. A mixture of stearin and palmitin was formerly called MARGARIN. As to the occurrence of palmitic acid, $C_{16}H_{32}O_2$, about the same remarks apply as to stearic acid. The mixture of these two acids has been called margaric acid, and this mixture occurs —often as very long, thin, crystalline plates—in old pus, in expectorations from gangrene of the lungs, etc.

Palmitin crystallizes, on cooling from a warm saturated solution in ether or alcohol, in starry rosettes of fine needles. The mixture of palmitin and stearin, called margarin, crystallizes, on cooling from a solution, as balls or round masses which consist of short or long, thin plates or needles which often appear like blades of grass. Palmitin, like stearin, has a variable melting- and solidifying-point, depending upon the way it has been previously treated. The melting-point is often given as 62° C., but some investigators[3] claim that it melts at 50.5° C., solidifies on further heating, and melts again at 66.5° C.

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[1] Annal. d. Chem. u. Pharm., **92**.

[2] Monatshefte f. Chem., **2**; see also Jaeckle, Zeitschr. f. physiol. Chem., **36**.

[3] R. Benedikt, Analyse der Fette, 3. Aufl., 1897, p. 44.

Palmitic acid, $\begin{array}{l} CH_3 \\ \cdot \\ (CH_2)_{14} \\ \cdot \\ COOH \end{array}$, crystallizes from an alcoholic solution in tufts of fine needles. It melts at 62° C.; still the admixture with stearic acid, as HEINTZ has shown, essentially changes the melting- and solidifying-points according to the relative amounts of the two acids. Palmitic acid is somewhat more soluble in cold alcohol than stearic acid; but they have about the same solubility in boiling alcohol, ether, chloroform, and benzene. Its barium salt contains 21.17 per cent barium, and silver salt contains 29.72 per cent silver.

Olein, or triolein, $C_{57}H_{104}O_6, = \begin{array}{l} CH_2.O.C_{18}H_{33}O \\ \cdot \\ CH.O.C_{18}H_{33}O \\ \cdot \\ CH_2.O.C_{18}H_{33}O \end{array}$, is present in all animal fats, and in greater quantities in vegetable fats. It is a solvent for stearin and palmitin. The oleic acid (elaic acid), $C_{18}H_{34}O_2$, as soaps, probably has about the same occurrence as the other fatty acids.

Olein is, at ordinary temperatures, a nearly colorless oil of a specific gravity of 0.914, without odor or marked taste, and solidifies in crystalline needles at −6° C. It becomes rancid quickly if exposed to the air. It dissolves with difficulty in cold alcohol, but more easily in warm alcohol or in ether. It is converted into its isomer, ELAÏDIN, by nitrous acid.

Oleic acid, $\begin{array}{l} CH_3 \\ \cdot \\ (CH_2)_7 \\ \cdot \\ CH \\ \cdot\cdot \\ CH \\ \cdot \\ (CH_2)_7 \\ \cdot \\ COOH \end{array}$, is an unsaturated acid of the series $C_nH_{n-2}O_2$, and correspondingly takes up two halogen atoms, i.e., iodine, at the double bondage, a factor which is the basis of v. HÜBL's method for determining the iodine equivalent. On taking up hydrogen, which can be accomplished by heating with hydroiodic acid and amorphous phosphorus, it is transformed into the corresponding saturated acid, namely, stearic acid. On oxidation the double bonds are satisfied by 2HO groups, and dioxystearic acid, $CH_3(CH_2)_7CHOH.CHOH(CH_2)_7COOH$, is formed. Oleic acid readily undergoes oxidation in the air with the formation of acid products, and the occurrence of monoxystearic acid, found in animal fats in certain instances, can be explained by this oxidation. Oleic acid on heating yields, besides volatile fatty acids, *sebacic acid,* $C_{10}H_{18}O_4$, which melts at 127°; and with nitrous acid it is transformed into its isomer, solid *elaidic acid,* which melts at 45° C.

Oleic acid forms at ordinary temperature a colorless, tasteless, and odorless oily liquid which solidifies in crystals at about 4° C., which

latter melt at 14° C. Oleic acid is insoluble in water, but dissolves in alcohol, ether, chloroform and petroleum ether. With concentrated sulphuric acid and some cane-sugar it gives a beautiful red or reddish-violet liquid whose color is similar to that produced in PETTENKOFER'S test for bile-acids. If a solution of oleic acid in glacial acetic acid is treated with a little chromic acid (in glacial acetic acid) and then with concentrated sulphuric acid, the green solution gradually becomes violet or cherry-red, and shows two characteristic absorption bands in the green, one a broad band near the blue and a second but fainter band near the yellow (LIFSCHÜTZ).[1] The barium salt of oleic acid contains 19.65 per cent barium and the silver salt 27.73 per cent silver.

If the watery solution of the alkali compounds of oleic acid is precipitated with lead acetate, a white, tough, sticky mass of lead oleate is obtained, which is not soluble in water and only slightly in alcohol, but is soluble in ether. This salt is more easily soluble in benzene than the lead salts of stearic and palmitic acids, and this behavior of the lead salts toward ether and benzene is made use of in separating oleic acid from the other fatty acids.

An acid related to oleic acid, DOEGLIC ACID, which is solid at 4° C., liquid at 16° C., and soluble in alcohol, is found in the blubber of the Balœna rostrata. According to BULL this acid is probably only a mixture of oleic acid and another acid—*gadoleic acid*, $C_{20}H_{38}O_2$, having a melting-point of +24.5° C., and occurring in cod-liver oil, herring oil and in whale blubber. In addition to this acid BULL found in cod-liver oil, besides myristic, palmitic, oleic and erucic acids, another acid, having the formula $C_{16}H_{30}O_2$. KURBATOFF [2] has demonstrated the presence of linoleic acid in the fat of the silurus, sturgeon, seal, and certain other animals. Drying fats have also been found by AMTHOR and ZINK [3] in hares, wild rabbits, wild boar, and mountain-cock.

To detect the presence of fat in an animal fluid or tissue the fat must first be shaken out or extracted with ether. After the evaporation of the ether the residue is tested for fat and fatty acids. The neutral fats are differentiated from the fatty acids by the acrolein test, and the fatty acids by the fact that their solution in a mixture of alcohol and ether previously made bluish-violet with tincture of alkanet, becomes red in color. In separating the fats from cholesterin and other non-saponifiable substances, as well as for the determination of the kind of the various fatty bodies, they are saponified with caustic alkali, alcoholic potash, or with sodium alcoholate. In regard to these operations, as well as the further investigation and the separation of the various fatty acids from each other, we must refer to completer hand-books.

In addition to the methods already suggested there are other chemical methods which are important in investigating fats. Besides ascertaining the melting- and congealing-point we also determine the following: 1. The *acid equivalent*, which is a measure of the amount of fatty acids in a fat, is determined by

[1] Zeitschr. f. physiol. Chem., **56**.

[2] Bull, Ber. d. d. chem. Gesellsch., **39**; Kurbatoff, Maly's Jahresb., **22**.

[3] Zeitschr. f. anal. Chem., **36**.

titrating the fat dissolved in alcohol-ether with N/10 alcoholic caustic potash, using phenolphthalein as indicator. 2. The *saponification equivalent*, which gives the milligrams of caustic potash uniting with the fatty acids in the saponification of 1 gram fat with N/2 alcoholic caustic potash. 3. REICHERT-MEISSL'S *equivalent*, which gives the quantity of volatile fatty acids contained in a given amount of neutral fat (5 grams). The fat is saponified, then acidified with mineral acid, and distilled, whereby the volatile fatty acids pass over; the distillate is then titrated with alkali. 4. *Iodine equivalent* is the quantity of iodine absorbed by a certain amount of the fat by addition. It is chiefly a measure of the quantity of unsaturated fatty acids, principally oleic acid or olein, in the fat. Other bodies, such as cholesterin, may also absorb iodine or halogens. The iodine equivalent is generally determined according to the method suggested by v. HÜBL. 5. The *acetyl equivalent* measures the quantity of those constituents of fats which contain OH groups, and is found by converting these bodies (oxyfatty acids, alcohols and others) into the corresponding acetyl ester by boiling them with acetic acid anhydride.

In the quantitative estimation of fats, the finely divided dried tissues or the finely divided residue from an evaporated fluid is extracted with ether, alcohol-ether, benzene, or any other proper extraction medium. The lecithin (phosphatides) and other bodies are dissolved by the various extraction media, hence the results for fats are too high. The most exact method for the estimation of fat seems to be the method suggested by KUMAGAWA and SUTO,[1] who give a complete review of the literature of the subject.

The fats are poor in oxygen, but rich in carbon and hydrogen. They therefore represent a large amount of chemical energy, and yield correspondingly large quantities of heat on combustion. They take first rank among the foods in this regard, and are therefore of very great importance in animal life. We will speak more in detail of this significance, also of fat formation and of the behavior of the fats in the body, in the following chapters.

Cholesterin and isocholesterin ester, which will be discussed in a subsequent chapter, as well as the following bodies, are closely related to the fats.

Spermaceti. In the living spermaceti or white whale there is found in a large cavity in the skull an oily liquid called spermaceti, which on cooling after death separates into a solid crystalline part ordinarily called SPERMACETI, and into a liquid, SPERMACETI-OIL. This last is separated by pressure. Spermaceti is also found in other whales and in certain species of dolphin.

The purified, solid spermaceti, which is called CETIN, is a mixture of esters of fatty acids. The chief constituent is the cetyl-palmitic ester mixed with small quantities of compound esters of lauric, myristic, and stearic acids with radicals of the alcohols, LETHAL, $C_{12}H_{25}.OH$, METHAL, $C_{14}H_{29}.OH$, and STETHAL, $C_{18}H_{37}.OH$.

Cetin is a snow-white mass shining like mother-of-pearl, crystallizing in plates, brittle, fatty to the touch, and which has a varying melting-point of 30° to 50° C., depending upon its purity. Cetin is insoluble in water, but dissolves easily in cold ether or volatile and fatty oils. It dissolves in boiling alcohol, but crystallizes on cooling. It is saponified with difficulty by a solution of caustic potash

in water, but with an alcoholic solution it saponifies readily, and the above-mentioned alcohols are set free.

Ethal or cetyl alcohol, $C_{16}H_{34}O. = (CH_2)_{14}$, which occurs in smaller quantities in beeswax, and was found by LUDWIG and v. ZEYNEK in the fat from dermoid cysts—though this is denied by AMESEDER,[1]—forms white, transparent, odorless, and tasteless crystals which are insoluble in water but dissolve easily in alcohol and ether. Ethal melts at 49.5° C.

SPERMACETI-OIL yields on saponification valeric acid, small amounts of solid fatty acids, and PHYSETOLEIC ACID. This acid, which has, like hypogæic acid, the composition $C_{16}H_{30}O_2$, occurs also, as found by LJUBARSKY,[2] in considerable amounts in the fat of the seal. It forms colorless and odorless needle-shaped crystals which easily dissolve in alcohol and ether and melt at 34° C.

BEESWAX may be treated here as concluding the subject of fats. It contains three chief constituents: (1) CEROTIC ACID, $C_{26}H_{52}O_2$,[3] which occurs as cetyl ether in Chinese wax and as free acid in ordinary wax. It dissolves in boiling alcohol and separates as crystals on cooling. The cooled alcoholic extract of wax contains (2) CEROLEIN, which is probably a mixture of several bodies, and (3) MYRICIN, which forms the chief constituent of that part of wax which is insoluble in warm or cold alcohol. Myricin consists chiefly of palmitic-acid ester of melissyl (myricyl) alcohol, $C_{30}H_{61}.OH$. This alcohol is a silky, shining, crystalline body melting at 85° C. DUNHAM[4] has found *carnaubic acid*, $C_{24}H_{48}O_2$, in a phosphatide from the ox kidney.

2. Phosphatides.

In close relation to the fats stands a group of esters containing nitrogen, phosphoric acid and fatty acid radicals. The representative of this group longest known is lecithin. This latter is an ester combination of a nitrogenous base, choline, with a fatty acid-glycerophosphoric acid, and THUDICHUM[5] has shown that a large number of more or less analogous bodies occur in the animal body, especially in the brain. All of these bodies have received the name *phosphatides*.

Those phosphatides which contain only one phosphoric acid radical in the molecule are called *monophosphatides*; those with two such radicals *diphosphatides*. The monophosphatides may contain one, two or more atoms of nitrogen in the molecule, and hence we differentiate between monamido- (P:N=1:1), diamido- (P:N=1:2), triamido- (P: N=1: 3) monophosphatides, etc. The lecithin group belongs to the monamido-monophosphatides. The diamidomonophosphatides have been found, besides in the brain, by THUDICHUM, in the bile (HAMMARSTEN), in the

[1] Ludwig and v. Zeynek, Zeitschr. f. physiol. Chem. **23**; Ameseder, *ibid.*, **52**.

[2] Journ. f. prakt. Chem. (N. F.), **57**.

[3] See Henriques, Ber. d. deutsch. chem. Gesellsch., **30**, 1415.

[4] Journ. of biol. Chem., **4**.

[5] J. L. W. Thudichum, Die chemische Konstitution des Gehirns des Menschen, etc., Tübingen, 1901.

yolk of the egg (STERN and THIERFELDER), and in muscles (ERLANDSEN), and seem to be widely distributed. A triamidomonophosphatide, called *neottin* by S. FRÄNKEL and BOLAFFIO,[1] has been isolated from the yolk of the egg, and according to THUDICHUM a tetramidomonophosphatide occurs in ox bile. Among the monoamidodiphosphatides (P:N=2:1) we must mention *cuorin*, which was discovered and studied by ERLANDSEN, and a phosphatide of the same type occurring in egg-yolk recently described by MACLEAN.[2] According to THUDICHUM non-nitrogenous phosphatides are also possible (in the brain). If this be true these bodies must not, for the present at least, be classified as phosphatides.

The phosphatides seem to be closely related to each other; they influence the solubility and precipitation properties of each other, and are generally precipitated as mixtures which are extremely difficult to separate into individual constituents. They are also amorphous, and readily oxidized, and it is easy to understand why their preparation in a pure state is so extremely difficult. Under these circumstances we have no sufficient guarantee as to their chemical individuality, and the descriptions of their properties and composition must be accepted with a certain reservation.

The phosphatides thus far investigated seem to be chiefly ester combinations between nitrogenous bases and fatty acid-glycerophosphoric acid. According to THUDICHUM phosphatides exist which contain no glycerin group. The fatty acids occurring in the phosphatides may be of different kinds. It seems that at least one oleic acid radical, or another still less saturated fatty acid, always occurs in the phosphatides. The phosphatides on this account always take up iodine. They are, as above stated, autooxidizable, taking up oxygen from the air and being readily changed (ERLANDSEN). They also give a beautiful reaction with PETTENKOFER'S bile-acid test. The nitrogenous base is generally choline; in certain cases their nature is not known, and in others the statements are somewhat contradictory.

The phosphatides are included in the group of lipoids, which are difficult to characterize from a chemical standpoint, because they are generally not soluble in water and because each phosphatide is dissolved by at least one of the ordinary solvents of the fats. Among themselves they may show quite a striking difference in behavior toward such solvents. For example, one may be insoluble in cold alcohol or ether and another soluble therein, and such differences are of importance in their preparation. They are generally all precipitated from their solution by acetone

[1] Hammarsten, Zeitschr. f. physiol. Chem., **36**; Stern and Thierfelder, *ibid.*, **53**; Erlandsen, *ibid.*, **51**; Fränkel (and Bolaffio), Biochem. Zeitschr., **9**.

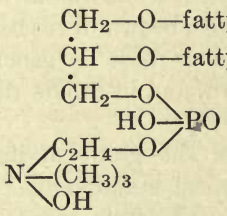
[2] Thudichum, Virchow's Arch., **156**; Erlandsen, l. c.; MacLean, Zeitschr. f. physiol. Chem., **57**.

although not completely, and this behavior is also of especial importance in their preparation. The phosphatides are also nearly all precipitated by metallic salts, especially by platinum chloride and cadmium chloride, and this method is also often used in their preparation. The usefulness of this method has been questioned at least for certain phosphatides, since ERLANDSEN showed that a decomposition occurs.

ERLANDSEN has also found that when finely divided heart-muscle, dried in the air, is completely extracted with ether and then with alcohol, the first extract contains the monophosphatides, and the alcohol extract contains the diamido phosphatides which were not free in the tissues, but existed in the combined state. Whether this observation is of general importance in the preparation of pure phosphatides remains to be seen.

In consideration of the uncertainty which exists as to the properties and chemical individuality of the various phosphatides, we will here only discuss in detail the most carefully studied phosphatides, namely, lecithin and cuorin. The others will be discussed in their proper place.

Lecithins. These bodies are ester compounds [1] of glycerophosphoric acid substituted by two fatty-acid radicals with a base called choline. According to the kind of fatty acid contained in the lecithin molecule it is possible to have various lecithins, such as stearyl-, palmityl-, and oleyl-lecithins. According to THUDICHUM [2] every true lecithin always contains at least one oleic-acid radical. According to the investigations of HENRIQUES and HANSEN, COUSIN and ERLANDSEN,[3] there is no question that the so-called lecithin of the egg-yolk and muscles must contain a fatty acid, still less saturated than oleic acid. All lecithins are monamidophosphatides, according to the following type:



The various lecithins stand close to each other in regard to constitution. The amount of phosphorus varies between 3.7–3.97 per cent and the amount of nitrogen between 1.7–1.9 per cent. The so-called di-

[1] Strecker, Annal. d. Chem. u. Pharm., **148**; Hundeshagen, Journ. f. prakt. Chem. (N. F.), **28**; Gilson, Zeitschr. f. physiol. Chem., **12**.

[2] Thudichum, Die chemische Konstitution des Gehirns des Menschen, etc., Tübingen, 1901.

[3] Henriques and Hansen, Skand. Arch. f. Physiol., **14** (1903); Cousin., Compt. rend., **137**; Erlandsen, Zetischr. f. physiol. Chem., **51**.

stearyl-lecithin studied by HOPPE-SEYLER and DIACONOW,[1] which probably has a different structure, has the formula $C_{44}H_{90}NPO_9$. ERLANDSEN gives the formula $C_{43}H_{80}NPO_9$ for the lecithin isolated by him from the heart muscles.

On saponification with alkalies or baryta-water, lecithin yields fatty acids, glycerophosphoric acid, and choline. It is only slowly decomposed by dilute acids. Besides small quantities of glycerophosphoric acid we have large quantities of free phosphoric acid split off. The lecithins are also decomposed by enzymes (lipase) with the splitting off of fatty acids.

Lecithin is optically active, and as the glycerophosphoric acid which can be split off is also active, WILLSTÄTTER and LÜDECKE [2] claim that the phosphoric acid is not bound on the middle unsymmetric CH group, but rather at the end CH_2 group of glycerin.

Lecithin, according to HOPPE-SEYLER,[3] is found in nearly all animal and vegetable cells thus far studied, and also in nearly all animal fluids. It is especially abundant in the brain, nerves, fish eggs, yolk of the egg, electrical organs of the Torpedo electricus, semen, and pus, and also in the muscles and blood corpuscles, blood plasma, lymph, milk, especially woman's milk, and bile. Lecithin is also found in different pathological tissues or liquids. As the presence of lecithin is only indirectly determined by the detection of phosphorous in organic combinations, it must be borne in mind that the above assertions relate chiefly to the occurrence of phosphatides.

The same also applies to the claims as to the quantity of lecithin in various organs and tissues as well as in different ages. In these cases the lecithin has not been prepared in a pure state, and the determinations represent only the approximate quantity of phosphatides. These determinations of SIWERTZOW, GLIKIN and NERKING,[4] show that lecithins (phosphatides) occur abundantly in the bone marrow, suprarenal capsule, heart and lungs, besides in the spinal marrow, brain, and egg, and also that the quantity varies strikingly in different varieties of animals. NERKING found 41.7 per cent lecithin in the bone marrow and 21.33 per cent in the suprarenal capsule of the urchin when calculated on the living organs, while the corresponding results in the rabbit were 2.71 and 2.39 per cent, respectively. These determinations have also shown that the amount of lecithin is especially higher in the new born, and that the phosphatides to all appearances are of the greatest importance

[1] Hoppe-Seyler, Med. chem. Unters., Heft **2** and **3**.

[2] Ber. d. d. chem. Gesellsch., **37**.

[3] Physiol. Chem. Berlin, 1877–81, p. 57.

[4] Siwertzow, see Biochem. Zeitschr., **2**, p. 310; Glikin, Biochem. Zeitschr., **4** and **7**; Nerking, *ibid.*, **10**.

in development. The new born come into the world with a store of phosphatides which diminishes during growth.

That the lecithins are of great importance in the development and growth of living organisms, in fact for the bioplastic processes in general, follows also from several investigations.[1] We have in lecithin or the phosphatides as a group, no doubt, very important material for the building up of the complicated phosphorized nuclein substances of the cell and cell nucleus. The wide distribution of the lecithins, as also the fact that they are primary cell constituents, gives great importance to these substances.

The statements as to the properties of the lecithins apply chiefly to the lecithin of the hen's egg, which since HOPPE-SEYLER and DIACONOW's time has been considered as distearyl-lecithin without any positive foundation. Other lecithin preparations correspond essentially with this, and certain differences between the various lecithins may be possibly due to decomposition products or to admixture with other phosphatides. It is still questioned whether the so-called distearyl-lecithin is a unit body or not.

Lecithin may be obtained in grains or warty masses composed of small crystalline plates by thoroughly cooling its solution in strong alcohol. In the dry state it has a waxy appearance, is plastic, but forms pulverizable masses when dried in vacuum, and is soluble in alcohol, especially on heating (to 40–50° C.); it is less soluble in ether. It is dissolved also by chloroform, carbon disulphide, benzene, and fatty oils. The solution of lecithin from egg-yolk is dextrorotatory (ULPIANI[2]). P. MAYER[3] claims to have prepared racemic lecithin from ordinary lecithin, and *l*-lecithin from the *r*-lecithin by cleavage with lipase. As he did not make use of pure lecithin it is difficult to judge his results. The solution of lecithin in alcohol-ether or chloroform is precipitated by acetone, although not completely. It swells in water to a pasty mass which shows under the microscope slimy, oily drops and threads, so-called myelin forms (see Chapter XII). On warming this swollen mass or the concentrated alcoholic solution, decomposition takes place with the production of a brown color. On allowing the solution or the swollen mass to stand, decomposition takes place and the reaction becomes acid. According to the investigations of LONG[4] the lecithins seem to be much more

[1] See Stoklasa, Ber. d. deutsch. chem. Gesellsch., **29**; Wiener Sitzungsber., **104**; Zeitschr. f. physiol. Chem., **25**; W. Danilewsky, Compt. rend., **121** and **123**, and W. Koch, Zeitschr. f. physiol. Chem., **37**; P. Kyes, *ibid.*, **41**, and Berl. klin. Wochenschr., 1904.

[2] Chem. Centralbl., 1901, **2**, p. 30 and 193.

[3] Biochem. Zeitschr., **1**.

[4] Journ. of Amer. chem. Soc., **30**, 1908.

resistant than was generally believed, and further investigations with pure lecithin are desirable.

With considerable water the lecithin gives an emulsion or colloidal solution which is not only precipitated by salts with divalent cations, Ca, Mg, and others as claimed by W. Koch, but is also precipitated according to Long and F. Gephart [1] by salts with monovalent cations, although slowly. In putrefaction lecithins yield glycerophosphoric acid and choline; the latter further decomposes with the formation of methylamine, ammonia, carbon dioxide, and marsh-gas (Hasebroek [2]). If dry lecithin be heated it decomposes, takes fire, and burns, leaving a phosphorized ash. On fusing with caustic alkali and saltpetre it yields alkali phosphates.

Lecithins combine with acids and bases. The compound with hydrochloric acid gives with platinum chloride a double salt which is insoluble in alcohol, soluble in ether, and which contains 10.2 per cent platinum (for distearyl-lecithin). The cadmium-chloride compound, which contains 3 molecules of lecithin and 4 molecules of cadmium chloride (Ulpiani [3]) is difficultly soluble in alcohol, but dissolves in a mixture of carbon disulphide and ether or alcohol. The molybdenum compound must also be mentioned (Ehrenfeld [4]). A solution of lecithin in alcohol is not precipitated by lead acetate and ammonia.

Lecithins are easily carried down during the precipitation of other compounds such as the protein bodies, and may therefore very greatly change the solubilities of other bodies. It is not known whether we are here dealing with an adsorption or a chemical combination, and the conditions are not the same in all cases. The combination with protein, the vitellines and lecithalbumins have been discussed in a previous chapter, and attention is there called to the necessity for more thorough investigation of this subject. Further investigations of the so-called lecithin-sugar (Bing) is also desirable, as we know nothing positive as to its nature. According to the investigations of Winterstein, Hiestand and E. Schulze,[5] lecithins (phosphatides) containing carbohydrates occur in the plant kingdom, and which contain about 20 per cent carbohydrate. We are still not decided whether we are here dealing with combinations or admixtures. The same is true for the iron content of the lecithins or phosphatides as observed by Glikin.[6]

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[1] W. Koch, Zeitschr. f. physiol. Chem., **37**; Long and Gephart, Journ. of Amer. Chem. Soc., **30**; see also Porges and Neubauer, Biochem. Zeitschr., **7**.

[2] Zeitschr. f. physiol. Chem., **12**.

[3] Chem. Centralbl., 1901, **2**, p. 30 and 193.

[4] Zeitschr. f. physiol. Chem., **56**.

[5] Winterstein and Hiestand, Zeitschr. f. physiol. Chem., **47** and **54**; Schulze, *ibid.*, **52** and **55**.

[6] Ber. d. d. chem. Gesellsch., **41**.

Various methods have been suggested by STRECKER, HOPPE-SEYLER and DIACONOW, THUDICHUM, GILSON, ZUELZER and BERGELL [1] for the preparation of the lecithins. As none of these yields a positively pure product we will only here mention them. According to ERLANDSEN'S experience all methods which are based upon the precipitation of the lecithin as a metallic compound should be avoided. The best method depends upon the solubility of the lecithin in alcohol and in ether in the cold and its precipitation by acetone (ERLANDSEN, H. E. ROAF and E. EDIE [2]). The work of ERLANDSEN is especially referred to in the preparation of lecithins.

For the present we have no quantitative method for estimating lecithin. The methods used in the past, when the amount of lecithin was calculated from the amount of phosphorus contained in the alcohol-ether extract is useless, as in this case the phosphorous content of all the phosphatides is determined and not alone of the lecithins. Even the detection of choline is not evidence, as this base probably also occurs in other phosphatides. In the detection of choline the double platinum compound is ordinarily prepared, and this can be done as described below. In special determinations of lecithin and cephalin KOCH used to heat with hydroiodic acid, and determined the methyl groups split off below 240° and those at about 300°. Instead of this he recommends with WOODS [3] to separate the two by precipitation in alcoholic solution, while boiling, with alcoholic lead acetate solution and a little ammonia, which precipitates only the cephalin.

Cephalin is also a monoamidophosphatide whose formula, based upon the investigations of THUDICHUM and KOCH,[4] is probably $C_{42}H_{82}NPO_{13}$. For the present cephalin must be classified with the lecithin group. The views of these two investigators as to the constitution of this body, which is difficult to purify, differ very considerably. According to THUDICHUM, on cleavage it yields neurine, glycerophosphoric acid, stearic acid, and a specific fatty acid, *cephalic acid*. According to KOCH it contains, on the contrary, only one methyl group attached to nitrogen, and is therefore probably dioxystearylmonomethyl lecithin, while according to COUSIN [5] it yields, like lecithin, stearic acid, an unsaturated fatty acid, glycerophosphoric acid and choline as decomposition products. Cephalin is amorphous and swells up in water like lecithin. It is soluble in cold ether, glacial acetic acid, and chloroform, but is insoluble in acetone and in alcohol, either cold or warm. The alcoholic solution, as previously

[1] Strecker, Annal. d. Chem. u. Pharm., **148**; Hoppe-Seyler and Diaconow, l. c.; Thudichum, l. c.; Gilson, Zeitschr. f. physiol. Chem., **12**; Zuelzer, *ibid.*, **27**; Bergell, Ber. d. d. chem. Gesellsch., **33**.

[2] Erlandsen, l. c.; Roaf and Edie, Thompson Yates Laboratory Reports, Vol. 6, part I, 1905.

[3] Koch and Woods, Journ. of biol. Chem., **1**.

[4] Thudichum, l. c.; Koch, Zeitschr. f. physiol. Chem., **36**.

[5] Compt. rend. soc. biol., **62**.

stated, is precipitated by an alcoholic lead acetate solution. Cephalin is obtained from the brain, after dehydration with acetone, by extracting with ether and precipitating the concentrated ethereal extract with alcohol. The cephalin is perhaps identical with the myeline substance isolated by ZUELZER [1] from the brain. The different statements as to the cleavage products of cephalin speak against its purity and chemical individuality.

Of the cleavage products of the lecithins choline is of especially great interest.

Choline (trimethyloxyethyl ammonium hydroxide),

$$C_5H_{15}NO_2, = HO.N\begin{cases} CH_2.CH_2.(OH) \\ (CH_3)_3, \end{cases}$$

stands in close relationship to the poisonous base *neurine* (trimethylvinyl ammonuim hydroxide), $HO.N\begin{cases} (CH_3)_3, \\ CH:CH_2 \end{cases}$, which according to BRIEGER can be formed from choline by the action of bacteria, and also to *muscarine*, $HO.N\begin{cases} (CH_3)_3 \\ CH_2CHO \end{cases}$, which is the aldehyde of choline and occurs in the fly agaric, and also to *betaine*, $H_3C.N\begin{cases} O \\ CH_2 \end{cases}CO$, which may be considered as the anhydride of the acid corresponding to choline. Muscarine and betaine can be obtained from choline on oxidation. Choline yields trimethylamine as decomposition product, and this seems to be formed in the transformation of choline in the animal body.

Choline occurs in the plant kingdom as well as in the animal kingdom. MOTT and HALLIBURTON have repeatedly found choline in the blood in degenerative diseases of the nervous system. It was first shown also in normal blood by MARINO ZUCO,[2] and this investigator first found it in the suprarenal capsule, but designated it neurine. LOHMANN found it later in this organ, and recently it has been found in various organs by other investigators, especially by C. SCHWARZ and v. FÜRTH. The fact that choline is a cleavage product of lecithin in the animal, and that it is antagonistic to adrenaline (of the suprarenal capsule) by its depressing action upon the blood pressure, and that it has an exciting action upon certain secretions (LOHMANN, THEISSIER and THÉVENOT, v. FÜRTH and SCHWARZ [3]), gives choline great physiological importance. It must

[1] Zeitschr. f. physiol. Chem., **27**.

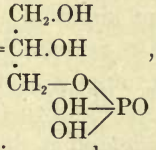
[2] Mott and Halliburton, Philos. Trans., Ser. B, **191** (1899) and **194** (1901); Marino Zuco, see Maly's Jahresber., **24**, pp. 181 and 698.

[3] Lohmann, Pflüger's Arch., **118** and **122**; v. Fürth and Schwarz, *ibid.*, **124**, which also contains the literature.

be remarked that MODRAKOWSKI [1] claims that the above action of choline does not exist in pure choline, but is due to a contamination with very small amounts of another substance having powerful action. Choline decomposes very readily and hence becomes easily contaminated. The choline isolated from tissues is readily contaminated, hence the claims made as to the physiological importance of choline in secretion require further confirmation.

Choline is a syrupy fluid, readily miscible with absolute alcohol. Hydrochloric acid gives with it a compound which is very soluble in water and alcohol, but insoluble in ether, chloroform, and benzene. This compound forms a double combination with platinum chloride, is soluble in water, insoluble in absolute alcohol and ether, and crystallizing ordinarily in six-sided orange-colored plates. This compound is used in the detection and identification of this base. Choline also forms a crystalline double compound with mercuric chloride and with gold chloride. Choline is precipitated by potassium iodide and iodine (GULEWITSCH), and potassium triiodide can be used for the quantitative estimation of this base (STANEK [2]). On heating the free base it decomposes into trimethylamine, ethylene oxide, and water.

In preparing choline from lecithins, and also for the detection of lecithin in an alcohol-ether extract, proceed as follows: The residue from the above or the solid lecithin is boiled one hour with baryta-water, filtered, and the excess of baryta precipitated by CO_2; filter while hot, concentrate to a syrup, and extract with absolute alcohol, when the insoluble barium glycerophosphate remains; then precipitate the filtrate with an alcoholic platinum chloride solution.

Glycerophosphoric acid, $C_3H_9PO_6$ =  , is a bibasic acid which probably occurs in the animal fluids and tissues only as a cleavage product of lecithins. According to WILLSTÄTTER and LÜDECKE [3] the glycerophosphoric acid split off from lecithins is optically active. Its barium and potassium salts are levorotatory, and behave in certain regards differently from the corresponding salts of synthetically prepared glycerophosphoric acid.

The diaminomonophosphatides have unfortunately been little studied, and the triamidomonophosphatide neottin will be discussed in a sub-

[1] Pflüger's Arch., **124**.

[2] Gulewitsch, Zeitschr. f. physiol. Chem., **24**; Stanek, *ibid.*, **56**. In regard to the quantitative estimation see also Kiesel, *ibid.*, **53**; Stanek, *ibid.*, **54**; Moruzzi, *ibid.*, **55**, and MacLean, *ibid.*, **55**.

[3] Ber. d. d. chem. Gesellsch., **37**.

sequent chapter (XIII). The only carefully studied monamidodiphosphatide is cuorin, discovered by ERLANDSEN.

Cuorin, $C_{71}H_{125}NP_2O_{21}$, is a monamidodiphosphatide prepared by ERLANDSEN[1] from the heart muscle of the ox, and which has an iodine equivalent of 101. It yields as cleavage products 3 molecules fatty acids of unknown nature but partly or entirely belonging to the series $C_nH_{2n-4}O_2$ and $C_nH_{2n-6}O_2$; also glycerin, phosphoric acid and a base which is not well known, but is not choline. Cuorin is autooxidizable, and gives PETTENKOFER'S bile acid test.

Cuorin is amorphous, yellowish-brown and similar to rosin. It gives a neutral solution with water which is like an emulsion. Cuorin does not reduce FEHLING'S solution, even after boiling with acids. It is soluble in ether, chloroform, petroleum ether and carbon disulphide. It dissolves with difficulty in benzene; it is insoluble in ethyl and methyl alcohol and in acetone. Cuorin is precipitated from its alcohol-ether solution by cadmium or platinum chloride.

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[1] Zeitschr. f. physiol. Chem., **51**, where the method of preparation is described.

CHAPTER VI.

THE BLOOD

The blood is to be considered from a certain standpoint as a fluid tissue; it consists of a transparent liquid, the *blood-plasma*, in which a vast number of solid particles, the *red* and *white blood-corpuscles* (and the *blood-plates*), are suspended. We also find in the blood granules of different kinds, which are to be considered as transformation products of the form-elements.[1]

Outside of the organism the blood, as is well known, coagulates more or less quickly; but this coagulation is accomplished generally in a few minutes after leaving the body. All varieties of blood do not coagulate with the same degree of rapidity. Some coagulate more quickly, others more slowly. In vertebrates with nucleated blood-corpuscles (birds, reptiles, batrachia, and fishes) Delezenne has shown that the blood coagulates very slowly if it is collected under such precautions that it does not come in contact with the tissues. On contact with the tissues or with their extracts it coagulates in a few minutes. The blood with non-nucleated blood-corpuscles (mammals), on the contrary, coagulates very rapidly. The coagulation of the blood in these cases may also be somewhat retarded by preventing the blood from coming in contact with the tissues (Spangaro, Arthus[2]). Among the varieties of blood of mammals thus far investigated the blood of the horse coagulates most slowly. The coagulation may be more or less retarded by quickly cooling; and if we allow equine blood to flow directly from the vein into a glass cylinder which is not too wide and which has been cooled, and let it stand at 0° C., the blood may be kept fluid for several days. An upper amber-yellow layer of plasma gradually separates from a lower red layer composed of blood-corpuscles with only a little plasma. Between these is observed a whitish-gray layer which consists of white blood-corpuscles.

The plasma thus obtained and filtered is a clear amber-yellow alkaline (toward litmus) liquid which remains fluid for some time when kept at 0° C., but soon coagulates at the ordinary temperature.

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[1] See Latschenberger, Wien. Sitzungsber , **105**.

[2] Delezenne, Compt. rend. Soc. de biol., **49**; Spangaro, Arch. ital. de Biol., **32**; Arthus, Journ. de Physiol. et Pathol., 4.

The coagulation of the blood may be prevented in other ways. After the injection of peptone, or, more correctly, proteose, solutions into the blood (in the living dog), it does not coagulate on leaving the veins (Fano, Schmidt-Mülheim [1]). The plasma obtained from such blood by means of centrifugal force is called *peptone-plasma*. According to Arthus and Huber [2] the caseoses and gelatoses act like fibrin proteose in dogs. Eel serum and certain lymph-forming extracts of organs (see Chapter VII) have an analogous action. The coagulation of the blood of warm-blooded animals is prevented by the injection of an effusion of the mouth of the officinal leech or a solution of the active substance of such an infusion, *hirudin* (Franz), into the blood current (Haycraft [3]). If the blood is allowed to flow directly, while stirring it, into a neutral salt solution—best a saturated magnesium-sulphate solution (1 vol. salt solution and 3 vols. blood)—we obtain a mixture of blood and salt which remains uncoagulated for several days. The blood-corpuscles, which, because of their adhesiveness and elasticity, would otherwise easily pass through the pores of the filter-paper, are made solid and stiff by the salt, so that they may be easily filtered off. The plasma thus obtained, which does not coagulate spontaneously, is called *salt-plasma*.

An especially good method of preventing coagulation of blood consists in drawing the blood into a dilute solution of potassium oxalate, so that the mixture contains 0.1 per cent oxalate (Arthus and Pagès [4]). The soluble calcium salts of the blood are precipitated by the oxalate, and hence the blood loses its coagulability. On the other hand, Horne [5] found that chlorides of calcium, barium, and strontium, when present in large amounts (2–3 per cent), may prevent coagulation for several days. According to Arthus [6] a non-coagulable blood-plasma may be obtained by drawing the blood into a sodium-fluoride solution until it contains 0.3 per cent NaFl.

On coagulation there separates in the previously fluid blood an insoluble or a very difficultly soluble protein substance, *fibrin*. When this separation takes place without stirring, the blood coagulates in a solid mass which, when carefully severed from the sides of the vessel, contracts, and a clear, generally yellow-colored liquid, the *blood-serum*, exudes. The solid coagulum which encloses the blood-corpuscles is called the

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[1] Fano, Arch. f. (Anat. u.) Physiol., 1881; Schmidt-Mülheim, *ibid.*, 1880.

[2] Arch. de Physiol. (5), 8.

[3] Haycraft, Proc. Physiol. Soc., 1884, 13, and Arch. f. exp. Path. u. Pharm., **18**; Franz, Arch. f. exp. Path. u. Pharm., **49**.

[4] Archives de Physiol. (5), **2**, and Compt. rend., **112**.

[5] Journ. of Physiol., **19**.

[6] Journ. de Physiol. et Pharm., **3** and **4**.

blood-clot (placenta sanguinis). If the blood is beaten during coagulation, the fibrin separates in elastic threads or fibrous masses, and the *defibrinated blood* which separates is sometimes called *cruor*,[1] and consists of blood-corpuscles and blood-serum, while uncoagulated blood consists of blood-corpuscles and blood-plasma. The essential chemical difference between blood-serum and blood-plasma is that the blood-serum does not contain even traces of the mother-substance of fibrin, the fibrinogen, which exists in the blood-plasma, while the serum is proportionally richer in another body, the fibrin ferment (see below).

I. BLOOD-PLASMA AND BLOOD-SERUM.

The Blood-plasma.

In the coagulation of the blood a chemical transformation takes place in the plasma. A part of the proteins separates as insoluble fibrin. The albuminous bodies of the plasma must therefore be first described. They are, as far as we know at present, *fibrinogen*, *nucleoprotein*, *serglobulins*, and *seralbumins*.

Fibrinogen occurs in blood-plasma, chyle, lymph, certain transudates and exudates, in bone-marrow (P. Müller), and perhaps also in other lymphoid organs. The seats of formation of fibrinogen are, according to Mathews, the leucocytes, especially of the intestine, according to Müller, the bone-marrow and probably other lymphoid organs such as the spleen and lymph glands, and according to Doyon and Nolf, the liver. The statement that the intestinal wall is a seat of formation of fibrinogen, a view that had already been held by Dastre, is substantiated not only by the direct researches of Mathews, but also by the older and substantiated opinion that the blood from the mesentery vein is richer in fibrinogen than the arterial blood. This origin of fibrinogen has been shown to be improbable by the recent researches of Doyon, Cl. Gautier and Morel. The occurrence of fibrinogen in the bone-marrow and other lymphoid organs as shown by Müller, and an increase of fibrinogen in the blood as well as in the bone-marrow of animals immunized with certain bacteria, especially pus-staphylococci, indicates the formation of fibrinogen in this tissue. The relation between the quantity of fibrin and leucocytosis as shown by many investigators such as Langstein and Mayer, Morawitz and Rehn, also indicate such a formation of fibrinogen. That the liver takes part in the formation of fibrinogen is implied by the fact that the quantity of fibrinogen in the blood

[1] The name *cruor* is used in different senses. We sometimes mean thereby only the blood when coagulated in a red solid mass, in other cases the blood-clot after the separation of the serum, and again the sediment consisting of red blood-corpuscles which is obtained from defibrinated blood by means of centrifugal force or by letting it stand.

strongly diminishes after the extirpation of the liver (Nolf), and that fibrinogen may indeed be entirely absent in the blood in phosphorus poisoning (Corin and Ansiaux, Jacoby, Doyon, Morel, and Kareff [1]), and that the blood of the hepatic vein, according to Doyon, Morel and Kareff,[2] is richer in fibrinogen than the blood from other vessels.

Fibrinogen has the general properties of the globulins, but differs from other globulins as follows: In a moist condition it forms white flakes which are soluble in dilute common salt solutions, and which easily conglomerate into tough, elastic masses or lumps. The solution in 5–10 per cent NaCl coagulates on heating at 52–55° C., and the faintly alkaline or nearly neutral weak salt solution coagulates at 56° C., or at exactly the same temperature at which the blood-plasma coagulates. Fibrinogen solutions are precipitated by an equal volume of a saturated common salt solution, and are completely precipitated by adding an excess of NaCl in substance (thus differing from serglobulin). A salt-free solution of fibrinogen in as little alkali as possible gives with $CaCl_2$ a precipitate which contains calcium and soon becomes insoluble. In the presence of NaCl or by the addition of an excess of $CaCl_2$ the precipitate does not appear.[3] A neutral solution of fibrinogen is precipitated by a concentrated solution of sodium fluoride when added in sufficient quantity. Fibrinogens from different kinds of blood behave somewhat differently in this regard. According to Huiskamp [4] fibrinogen from horse-blood hardly dissolves in NaCl of 3–5 per cent at ordinary temperatures, while it does dissolve at 40–45°. It also dissolves in ammonia of 0.05 per cent, and on the addition of 3–5 per cent NaCl this solution can be neutralized. The fibrinogen prepared by Huiskamp in this way retained its typical properties. Fibrinogen differs from the myosin of the muscles, which coagulates at about the same temperature, and from other protein bodies in the property of being converted into fibrin under certain conditions. Fibrinogen has a strong decomposing action on hydrogen peroxide. It is quickly made insoluble by precipitation with water or with dilute acids. Its specific rotation is $(\alpha)_D = -52.5°$ according to Mittelbach.[5]

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[1] P. Müller, Hofmeister's Beiträge, **6**; Mathews, Amer. Journ. of Physiol., **3**; Nolf, Bull. Acad. Roy. Belg., 1905, and Arch. intern. de Physiol., **3**, 1905; Langstein and Mayer, Hofmeister's Beiträge, **5**; Morawitz and Rehn, Arch. f. exp. Path. u. Pharm., **58**; Corin and Ansiaux, Maly's Jahresber., **24**; Jacoby, Zeitschr. f. physiol. Chem., **30**; Doyon, Morel, and Kareff, Compt. rend., **140**; Doyon, Morel, and Péju, Comp. rend. soc. biolog., **58**; Doyon, Cl. Gautier and Morel, *ibid.*, **62**.

[2] Journ. de Physiol., 8 (1906).

[3] See Hammarsten, Zeitschr. f. physiol. Chem., **22**; Cramer, *ibid.*, **23**.

[4] Huiskamp, *ibid.*, **44** and **46**. In regard to fibrinogen the reader is referred to the author's investigations. Pflüger's Archiv, **19** and **22**, and Zeitschr. f. physiol. Chem., **28**.

[5] Zeitschr. f. physiol. Chem., **19**.

Fibrinogen may be easily separated from the salt-plasma or oxalate-plasma by precipitation with an equal volume of a saturated NaCl solution. It must be observed that the oxalate-plasma can only be employed after the precipitate, containing proenzymes, and produced by exposure to cold, has settled and been filtered off. If this is not done then the fibrinogen is always impure. A neutralization of the plasma is not necessary, and is not to be recommended. For further purification the precipitate is pressed, redissolved in an 8-per cent salt solution, the filtrate precipitated by a saturated salt solution as above, and after being treated in this way three times, the precipitate at last obtained is pressed between filter-paper and finely divided in water. The fibrinogen dissolves with the aid of the small amount of NaCl contained in itself, and the solution may be made salt-free by dialysis with very faintly alkaline water. The fibrinogen can be nearly freed from fibrin-globulin, which will be spoken of later, by precipitating with double the volume of saturated sodium-fluoride solution, redissolving in water with 0.05-per cent ammonia, and then neutralizing this solution, treated with NaCl, and repeating this several times. Fibrinogen may also, according to Reye,[1] be prepared by fractionally precipitating the plasma with a saturated solution of ammonium sulphate. We have no knowledge as to the purity of the fibrinogen so prepared. From transudates we ordinarily obtain a fibrinogen which is strongly contaminated with lecithin and which can hardly be purified without decomposing it. The methods for the detection and quantitative estimation of fibrinogen in a liquid were formerly based on its property of yielding fibrin on the addition of a little blood, of serum, or of fibrin ferment. Reye has suggested the fractional precipitation with ammonium sulphate as a quantitative method. The value of this method has not been sufficiently tested.

Fibrinogen stands in close relation to its transformation product, fibrin.

Fibrin is the name of that protein body which separates on the so-called spontaneous coagulation of blood, lymph, and transudates as well as in the coagulation of a fibrinogen solution after the addition of serum or fibrin ferment (see below).

If the blood is beaten during coagulation, the fibrin separates in elastic, fibrous masses. The fibrin of the blood-clot may be beaten to small, less elastic, and not particularly fibrous, lumps. The typical fibrous and elastic white fibrin, after washing, stands, in regard to its solubility, close to the coagulated proteins. It is insoluble in water, alcohol, or ether. It expands in hydrochloric acid of 1 p. m., as also in caustic potash or soda of 1 p. m., to a gelatinous mass, which dissolves at the ordinary temperature only after several days; but at the temperature of the body it dissolves more readily, although still slowly. Fibrin may be dissolved by dilute salt solutions after a long time at the ordinary tem-

[1] W. Reye, Ueber Nachweis und Bestimmung des Fibrinogens, Inaug.-Diss. Strassburg, 1898.

perature, or much more readily at 40° C., and this solution takes place, according to Arthus and Hubert and also Dastre,[1] without the aid of micro-organisms. This action is due to proteolytic enzymes carried down by the fibrin or enclosed within the leucocytes (Rulot[2]). According to Green and Dastre[3] two globulins are formed in the solution of fibrin in neutral salt solution, and according to Rulot also proteoses (and peptones) on the solution of fibrin containing leucocytes. Fibrin, like fibrinogen, decomposes hydrogen peroxide, due to a contamination with catalases, but this property is destroyed by heating or by the action of alcohol.

What has been said of the solubility of fibrin relates only to the typical fibrin obtained from the arterial blood of oxen or man by whipping and washing first with water and with common salt solution, and then with water again. The blood of various kinds of animals yields fibrin with somewhat different properties, and according to Fermi[4] pig-fibrin dissolves much more readily than ox-fibrin in hydrochloric acid of 5 p. m. Fibrins of varying purity or originating from blood from different parts of the body have unlike solubilities.

The fibrin obtained by beating the blood, and purified as above described, is always contaminated by secluded blood-corpuscles or remains thereof, and also by lymphoid cells. It can be obtained pure only from filtered plasma or filtered transudates. For the preparation of pure fibrin, as well as for the quantitative estimation of it, the spontaneously coagulating liquid is at once, or the non-spontaneously coagulating liquid only after the addition of blood-serum or fibrin ferment, thoroughly beaten with a whalebone, and the separated coagulum is washed first in water and then with a 5-per cent common salt solution, and again with water, and finally extracted with alcohol and ether. If the fibrin is allowed to stand for some time in contact with the blood from which it was formed, it partly dissolves (*fibrinolysis*—Dastre[5]). This fibrinolysis must be prevented in the exact quantitative estimation of fibrin (Dastre). The blood constituents that are active in fibrinolysis are still unknown, but they are without doubt of enzymotic nature. It must be mentioned that a strong fibrinolysis takes place in blood after acute phosphorus-poisoning (Jacoby and others), after extirpation of the liver (Nolf), and also when the coagulability of the blood has been reduced by the injection of proteoses (Nolf, Rulot[6]).

A pure fibrinogen solution may be kept at the ordinary temperature until putrefaction begins without showing a trace of fibrin coagula-

[1] Arthus and Hubert, Arch. de Physiol. (5), **5**; Dastre, *ibid.*, (5) **7**.

[2] Arch. intern. de Physiol., **1**.

[3] Green, Journ. of Physiol., 8; Dastre, l. c.

[4] Zeitschr. f. Biologie, 28.

[5] Archives de Physiol. (5), **5** and **6**.

[6] Jacoby, Zeitschr. f. physiol. Chem., **30**; Nolf, Arch. intern. de Physiol., **3**, 1905; Rulot, l. c.

tion. But if to this solution is added a water-washed fibrin-clot or a little blood-serum, it immediately coagulates, and may yield perfectly typical fibrin. The transformation of the fibrinogen into fibrin requires the presence of another body contained in the blood-clot and in the serum. This body, whose importance in the coagulation of fibrin was first observed by BUCHANAN,[1] was later rediscovered by ALEXANDER SCHMIDT,[2] and designated as *fibrin ferment* or *thrombin*. The nature of this enzymotic body has not been ascertained with certainty. Although many investigators, especially English, consider fibrin ferment as a globulin, still more recent experiments of PEKELHARING and others show that it is a nucleoprotein which, according to HUISKAMP,[3] occurs in the thymus gland partly as nucleohistone and partly in another form. Fibrin ferment is produced, according to PEKELHARING, by the influence of soluble calcium salts on a preformed zymogen existing in the non-coagulated plasma. SCHMIDT admits the presence of such a mother-substance of the fibrin ferment in the blood, and calls it *prothrombin*. The conversion of this mother-substance into thrombin is a very complicated process, which will be discussed under the coagulation of the blood. Thrombin is like other enzymes in that the very smallest amount of it produces an action, and its solution becomes inactive on heating. The velocity of coagulation is dependent upon the quantity of thrombin, and FULD has found that at least within certain limits an increase of double the quantity of enzyme causes an increase in speed of coagulation of one and one-half. This closely corresponds to SCHULTZ's law; but it is true only for experiments with bird plasma and tissue extracts. MARTIN[4] has found another law from experiments with plasma and snake-poisons containing thrombin. According to him the behavior is as follows: As in the casein coagulation with rennin, the celerity of coagulation is inversely proportional to the quantity of ferment; and LOEB has observed a similar conduct with invertebrates. The optimum of the thrombin action lies at about 40° C.; at 70–75° C. the enzyme is destroyed in neutral solution. The question as to whether the thrombin found in different animals is the same substance or whether we have several thrombins, has not been decided. The latter is not improbable; neverthe-

[1] London Med. Gazette, 1845, 617. Cit. by Gamgee, Journal of Physiol., 1879.

[2] Pflüger's Arch., **6**; see also Zur Blutlehre, 1892, and Weitere Beiträge zur Blutlehre, 1895.

[3] Pekelharing, Verhandl. d. Kon. Akad. d. Wetensch. te Amsterdam, 1892, Deel 1; *ibid.*, 1895, and Centralbl. f. Physiol., **9**; Wright, Proc. Roy. Irish Acad. (3), **2**; The Lancet, 1892, and On Wooldridge's Method, etc., British Med. Journal, 1891; Lilienfeld, Hämatol. Untersuch. Arch. f. (Anat. u.) Physiol., 1892; Ueber Leukocyten und Blutgerinnung, *ibid.*; Halliburton and Brodie, Journal of Physiol., **17** and **18**; Huiskamp, Zeitschr. f. physiol. Chem., **32**; Pekelharing and Huiskamp, *ibid.*, **39**.

[4] Martin, Journ. of Physiol., **32**; Hofmeister's Beiträge, **2**; Loeb, *ibid.*, **9**.

less a definite specificity of different thrombins has not been observed with certainty.

The isolation of thrombin has been tried in several ways. Ordinarily it may be prepared by the following method, proposed by ALEX. SCHMIDT:[1] Precipitate the serum or defibrinated blood with 15–20 vols. of alcohol and allow it to stand a few months. The precipitate is then filtered off and dried over sulphuric acid. The ferment may be extracted from the dried powder by means of water. Other methods have been suggested by HAMMARSTEN and by PEKELHARING.[2]

The preparation of a thrombin solution as free as possible from lime may be accomplished by removing the lime salts from the serum by means of an oxalate and precipitating the serum with alcohol and allowing it to stand under alcohol for several months. The dried powder is rubbed with water, and freed from soluble salts by repeated lixiviation with water and by the use of centrifugal force. Then each gram of powder is allowed to stand some time with 100–150 cc. water, is filtered, and in this way a solution is obtained which contains only about 0.3–0.4 p. m. solids and about 0.0007 p. m. CaO (HAMMARSTEN).

If a fibrinogen solution containing salt, as above prepared, is treated with a solution of thrombin, it coagulates at the ordinary temperature more or less quickly and yields a typical fibrin. Besides the thrombin, the presence of neutral salts is necessary, for ALEX. SCHMIDT has shown that fibrin coagulation does not take place without them. The presence of soluble calcium salts is not, as is generally assumed, a positive condition for the formation of fibrin, because, as shown by ALEX. SCHMIDT, PEKELHARING, and HAMMARSTEN,[3] thrombin can transform fibrinogen into typical fibrin in the absence of lime salts precipitable by oxalate. The fibrin is not richer in lime than the fibrinogen used in its preparation if the fibrinogen and thrombin solutions are employed as lime-free as possible, and the view that the fibrin formation is connected with a taking up of lime has been shown to be untenable (HAMMARSTEN). The quantity of fibrin obtained on coagulation is always smaller than the amount of fibrinogen from which the fibrin is derived, and we always find a small amount of protein substance in the solution. It is therefore not improbable that the fibrin coagulation, in accordance with the views first proposed by DENIS, is a cleavage process in which the soluble fibrinogen is split into an insoluble protein, the fibrin, which forms the chief mass, and a soluble protein substance which is produced only in small amounts. We find a globulin-like substance which coagulates at about 64° C. in blood-serum as well as in the serum from coagulated fibrinogen solutions.

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[1] Pflüger's Arch., 6.

[2] Hammarsten, *ibid.*, 18; Pekelharing, l. c.

[3] See Hammarsten, Zeitschr. f. physiol. Chem., 22, which also cites the works of Schmidt and Pekelharing, and *ibid.*, 28.

This substance is called *fibrin-globulin* by HAMMARSTEN. The recent investigations of HUISKAMP have shown that this substance is not formed as a cleavage product from pure fibrinogen, but occurs in plasma or in fibrinogen solutions not purified of sodium fluoride besides the fibrinogen, or perhaps in loose combination with fibrinogen. The view that a cleavage takes place in the coagulation of the fibrinogen has not been supported by these investigations.[1]

Opinions are not unanimous in regard to the enzyme nature of thrombin and the enzymotic formation of fibrin, and there are, indeed, investigators who consider the coagulation as a physical process or a reaction between colloids (ISCOVESCO, NOLF and others[2]). A more thorough discussion of this subject can take place only in connection with the coagulation of the blood.

Nucleoprotein. This substance, which, as above-mentioned, is considered by PEKELHARING and HUISKAMP as identical with the prothrombin or thrombin, occurs in the blood-plasma as well as in the serum, and is precipitated from the latter with the globulin. It is similar to the globulin in that it is readily soluble in neutral salt solution, and can be completely salted out on saturation with magnesium sulphate, and separates only incompletely on dialysis. It is much less soluble than serglobulin in an excess of dilute acetic acid, and coagulates at 65–69° C. C. G. LIEBERMEISTER[3] found only 0.08–0.09 per cent phosphorus in the nucleoprotein, which indicates that the nucleoprotein was contaminated with other proteins. He also found that the substance was soluble in acetic acid with difficulty, a property which is used by PEKELHARING as an important means of separating the compound proteins from the globulins.

Serglobulins, also called *paraglobulin* (KÜHNE), *fibrinoplastic substance* (ALEX. SCHMIDT), *serum-casein* (PANUM[4]), occur in the plasma, serum, lymph, transudates and exudates, in the white and red corpuscles, and probably in many animal tissues and form-elements, though in small quantities. They are also found in the urine in many diseases.

The so-called serglobulin is without doubt not an individual substance, but consists of a mixture of two or more protein bodies which cannot be completely and positively separated from each other. The mixture of globulins obtained from blood-plasma or blood-serum by saturation with magnesium sulphate or half-saturation with ammonium sulphate consists of nucleoprotein, fibrin-globulin, and the true serglobulin or mixture of globulins.[5]

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[1] See Hammarsten, Zeitschr. f. physiol. Chem., **28**; Heubner, Arch. f. exp. Path. u. Pharm., **49**, and Zeitschr. f. physiol. Chem., **45**; Huiskamp, *ibid.*, **44** and **46**.

[2] Iscovesco, Compt. rend. soc. biol., **60** and **61**; Nolf, Arch. internat. d. Physiol., **6** (1908).

[3] Hofmeister's Beiträge, 8; Pekelharing and Huiskamp, l. c. footnote 3, page 248.

[4] Kühne, Lehrbuch d. physiol. Chem., Leipzig, 1866–68; Alex. Schmidt, Arch. f. (Anat. u.) Physiol., 1861–62; Panum, Virchow's Arch., **3** and **4**.

[5] Mellanby, Journ. of Physiol., **36**, claims that no separation of the two chief

The nucleoprotein has already been discussed. The fibrin-globulin, which occurs in the serum only in small amounts, can be completely precipitated by NaCl. It has the general properties of the globulins, but differs from the serglobulins by a lower coagulation temperature, 64–66° C., and also in that it is precipitated by $(NH_4)_2SO_4$ even at 28 per cent saturation.

Serglobulins. If the globulin obtained by saturation with magnesium sulphate is dialyzed, then, as has been known for a long time and further substantiated by MARCUS, only a part of the globulin separates out, while a portion remains in solution and cannot be precipitated by the addition of acid. For this reason MARCUS [1] also differentiates between a water-soluble globulin and one insoluble in water. According to the recent investigations of HOFMEISTER and PICK [2] the part insoluble in water corresponds chiefly to a globulin fraction readily precipitated by $(NH_4)_2SO_4$ (by 28–36 vols. per cent saturated solution), and the part soluble in water corresponds to a more difficultly precipitable fraction (by 36–44 vols. per cent saturated solution). The first fraction is called *euglobulin* and the second *pseudoglobulin*. According to PORGES and SPIRO [3] the serglobulins can be separated by $(NH_4)_2SO_4$ into three fractions whose precipitation limits are 28–36, 33–42, and 40–46 vols. per cent saturated solution. All three fractions contain globulin insoluble in water. FREUND and JOACHIM [4] have found that the euglobulin as well as the pseudoglobulin fraction is a mixture of globulin soluble in water and globulin insoluble in water, and consequently the number of different globulins in the serum may be still greater.

It follows from all these investigations that either the difference between the globulin soluble in water and that insoluble is not sufficient or that the fractional precipitation with ammonium sulphate is not suited for the separation of the various globulins. This latter seems to be the case, as shown by HASLAM [5]. It must not be forgotten that the globulin fractions are always contaminated with other serum constituents, and that these may influence the solubility and precipitability. As HAMMARSTEN has shown, a water-soluble globulin can be transformed into a globulin insoluble in water by careful purification, and also the reverse, namely, a globulin insoluble in water can sometimes be converted into one soluble in water by allowing it to lie in the air. An insoluble protein like casein can also, according to HAMMARSTEN,[6] have the solubilities of a globulin due to contamination with constituents of the serum, and K. MÖRNER [7] has also

groups of proteins, the globulins and albumins can be accomplished by saturation with $MgSO_4$ of half-saturation with $(NH_4)_2SO_4$.

[1] Zeitschr. f. physiol. Chem., **28**.

[2] Hofmeister's Beiträge, **1**,

[3] Hofmeister's Beiträge, **3**.

[4] Zeitschr. f. physiol. Chem., **36**.

[5] Journ. of Physiol., **32**.

[6] See Hammarsten, Ergebnisse d. Physiol., **1**, Abt. **1**.

[7] Zeitschr. f. physiol. Chem., **34**.

shown that a contamination of the serum-globulins with soap can essentially modify the precipitation of these globulins. Under these circumstances the above assumptions in regard to the different globulin fractions must be accepted with great caution.

The investigations made thus far upon the so-called serglobulin have not led to any positive results. That this globulin, with the exception of the enzymes, immune bodies, and other unknown substances which are carried down by the various fractions, is a mixture of globulins there seems to be no doubt. The serglobulin or the globulin mixture which is obtained from the serum by the methods to be described has the following properties:

In a moist condition it forms snow-white flaky masses, neither tough nor elastic, which always contain thrombin and hence can bring about coagulation in a fibrinogen solution. The neutral solution is only incompletely precipitated by NaCl added to saturation, and is not precipitated by an equal volume of a saturated salt solution. It is only partly precipitated by dialysis or by the addition of acid. On saturation with magnesium sulphate or one-half saturation with ammonium sulphate a complete precipitation is obtained. The coagulation temperature is, with 5–10 per cent NaCl in solution, 69–76°, but more often 75° C. The specific rotation of the solution containing salt is $(\alpha)_D = -47.8°$ for the serglobulin from ox-blood (Fredericq [1]). The various globulin fractions do not differ essentially from each other in their coagulation temperatures, specific rotation, refraction coefficient (Reiss [2]), and their elementary composition. The average composition is, according to Hammarsten, C 52.71, H 7.01, N 15.85, S 1.11 per cent. K. Mörner [3] found 1.02 per cent sulphur and 0.67 per cent lead-blackening sulphur. All the sulphur seems to exist as cystine.

Serglobulin contains, as K. Mörner first showed, a carbohydrate group which can be split off. Langstein [4] has obtained several carbohydrates from the blood-globulin, namely, dextrose, glucosamine, and carbohydrate acids of unknown kinds. It has not been shown whether these small amounts of carbohydrate are derived from the globulin or from other contaminating bodies. According to Zanetti and also Bywaters, the blood-serum contains a glucoproteid, *seromucoid*, and the investigations of Eichholz [5] seem to show that the globulins are

[1] Bull. Acad. Roy. de Belg. (2), **50**. In regard to paraglobulin, see Hammarsten, Pflüger's Arch., **17** and **18**, and Ergebnisse d. Physiol., **1**, Abt. 1.

[2] Hofmeister's Beiträge, **4**.

[3] Zeitschr. f. physiol. Chem., **34**.

[4] Mörner, Centralbl. f. Physiol., **7**; Langstein, Münch. med. Wochenschr., 1902, 1876, and Wien. Sitzungsber., **112**, Abt. II*b*, 1903; Monatsheft f. Chem., **25**; Hofmeister's Beiträge, **6**; see also footnote 3, p. 83.

[5] Zanetti, Chem. Centralbl., 1898, I, p. 624; Bywaters, Journ. of Physiol., **35**, and Bicohem. Zeitschr., **15**; Eichholz, Journ. of Physiol., **23**.

contaminated by a glucoproteid. According to LANGSTEIN the sugar is not only mixed with the globulin, but it exists in a combined form, probably in loose combination.

Serglobulin (the euglobulin) may be easily separated as a fine flocculent precipitate from blood-serum by neutralizing or making faintly acid with acetic acid and then diluting with 10–20 vols. of water. For further purification this precipitate is dissolved in dilute common salt solution, or in water with the aid of the smallest possible amount of alkali, and then reprecipitated by diluting with water or by the addition of a little acetic acid. All the serglobulin may also be separated from the serum by means of magnesium or ammonium sulphate; in these cases it is difficult to completely remove the salt by dialysis. As long as we are not agreed as to the number of globulins in the serum, it is not necessary to give a method of separating the various globulins in this mixture. Thus far the fractional precipitation with $(NH_4)_2SO_4$ has been used chiefly. The serglobulin from blood-serum is always contaminated with lecithin and thrombin. A serglobulin free from thrombin may be prepared from ferment-free transudates, as sometimes from hydrocele fluids, and this shows that serglobulin and thrombin are different bodies. For the detection and the quantitative estimation of serglobulin we may use the precipitation by magnesium sulphate added to saturation (HAMMARSTEN), or by an equal volume of a saturated *neutral* ammonium-sulphate solution (HOFMEISTER and KAUDER and POHL [1]). In the quantitative estimation the precipitate is collected on a weighed filter, washed with the salt solution employed, dried with the filter at about 115° C., then washed with boiling-hot water, so as to completely remove the salt, extracted with alcohol and ether, dried, weighed, and incinerated to determine the ash. The accuracy of these methods is questionable, as shown by the researches of HASLAM.

Seralbumins are found in large quantities in blood-serum, blood-plasma, lymph, transudates, and exudates. Probably they also occur in other animal fluids and tissues. The proteins which pass into the urine under pathological conditions consist largely of seralbumin.

The seralbumin, like the serglobulin, seems also to be a mixture of at least two protein bodies. The preparation of crystalline seralbumin (from horse-serum) was first performed by GÜRBER. It crystallizes with difficulty from other blood-sera (GRUZEWSKA). Even from horse-serum only a portion of the albumins is obtained as crystals, and it is also possible that the amorphous albumin, which is precipitated by ammonium sulphate with difficulty, represents two seralbumins (MAXIMOWITSCH). According to GÜRBER and MICHEL it would seem that the crystalline serabumin is also a mixture, but this is disproved by the observations of SCHULZ, WICHMANN, and KRIEGER.[2] We know nothing as to the

[1] Hammarsten, l. c.; Hofmeister, Kauder and Pohl, Arch. f. exp. Path. u. Pharm., **20**.

[2] In regard to the literature on the crystalline seralbumins, see Schulz, Die Kristallisation von Eiweissstoffen, Jena, 1901; Maximowitsch, Maly's Jahresber., **31**, 35.

behavior of the amorphous fraction of the seralbumin in this respect. Because of the different coagulation temperatures, HALLIBURTON claims the existence of three different albumins in the blood-serum, a view which has been disputed by several experimenters, and recently by HOUGARDY. On the other hand, the earlier investigations of KAUDER, as well as the more recent work of OPPENHEIMER,[1] seem to indicate a non-unit nature of the seralbumins, but this question is still an open one.

The crystalline seralbumin may perhaps be a combination with sulphuric acid (K. MÖRNER, INAGAKI). The coagulated albumin obtained from the aqueous solution of the crystals with the aid of alcohol has nearly the same elementary composition (MICHEL) as the amorphous mixture of albumin prepared from horse-serum (HAMMARSTEN and K. STARKE[2]). The average composition was C 53.06, H 6.98, N 15.99, S 1.84 per cent. K. MÖRNER, after the removal of the sulphuric acid from crystalline albumin, found 1.73 per cent total sulphur, which probably exists only as cystine. LANGSTEIN[3] has been able to split off a nitrogenous carbohydrate (glucosamine) from crystalline seralbumin. The quantity was so small that the question is still undecided whether or not the carbohydrate was a contamination. The fact that ABDERHALDEN, BERGELL, and DÖRPINGHAUS[4] were able to prepare a seralbumin entirely free from carbohydrate and which did not respond to MOLISCH'S very delicate reaction, seems to be decisive on this point. The specific rotation of crystalline seralbumins from horse-serum was found by MICHEL to be $(\alpha)_D = -61\text{–}61.2°$, and by MAXIMOWITSCH on the contrary $(\alpha)_D = -47.47°$.

The crystalline and amorphous seralbumin in aqueous solution give the ordinary albumin reactions. The coagulation temperature of a 1-per cent solution poor in salts is about 50° C., but rises with the quantity of salt. The coagulation of the mixture of albumins from serum generally takes place at 70–85° C., but is essentially dependent upon the reaction and the amount of salt present. Up to the present time no seralbumin solution has been prepared free from mineral bodies. A solution as free from salts as possible does not coagulate either on boiling or on the addition of alcohol. On the addition of a little common salt it coagulates in both cases.[5]

[1] Halliburton, Journ. of Phyisol., **5** and **7**; Hougardy, Centralbl. f. Physiol., **15**, 665; Oppenheimer, Verhandl. d. physiol. Gesellsch., Berlin, 1902.

[2] Michel, Verhandl. d. phys.-med. Gesellsch. zu Würzburg, **29**, No. 3; K. Starke, Maly's Jahresber., **11**; K. Mörner, l. c.; Inagaki, Biochem. Centralbl., **4**, p. 515.

[3] K. Mörner, l. c.; Langstein, Hofmeister's Beiträge, **1**.

[4] Zeitschr. f. physiol. Chem., **41**.

[5] In regard to the relationship of neutral salts to heat coagulation, see J. Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München, 1897.

Seralbumin differs from the albumin of the white of the hen's egg in the following particulars: It is more levogyrate; the precipitate formed by hydrochloric acid easily dissolves in an excess of the acid; it is rendered less insoluble by alcohol.

In preparing the seralbumin mixture, first remove the globulins, according to JOHANSSON, by saturating with magnesium sulphate at about 30° C. and filtering at the same temperature. The cooled filtrate is separated from the crystallized salt and is treated with acetic acid so that it contains about 1 per cent. The precipitate formed is filtered off, pressed, dissolved in water with the addition of alkali to neutral reaction, and the solution freed from salt by dialysis. The mixture of albumins may be obtained in a solid form from the dialyzed solution either by evaporating the solution at a gentle temperature or by precipitating with alcohol, which must be quickly removed. STARKE [1] has suggested another method, which is also to be recommended. The crystalline seralbumin may be prepared from serum freed from globulin by half saturating with ammonium sulphate, by the addition of more salt until a cloudiness appears, and then proceeding according to the suggestion of GÜRBER and MICHEL. On acidification with acetic acid or sulphuric acid the crystallization may be considerably accelerated.[2] In the detection and quantitative estimation of seralbumin, the filtrate from the globulin precipitated with magnesium sulphate can be heated to boiling, after acidifying with a little acetic acid if necessary. The quantity of seralbumin is best calculated as the difference between the total proteins and the globulin.

Summary of the elementary composition of the above-mentioned and described proteins (from horse-blood):

	C	H	N	S	O	
Fibrinogen	52.93	6.90	16.66	1.25	22.26	(HAMMARSTEN)
Fibrin	52.68	6.83	16.91	1.10	22.48	"
Fibrin-globulin	52.70	6.98	16.06	....	.....	"
Serglobulin	52.71	7.01	15.85	1.11	23.32	"
Seralbumin	53.08	7.10	15.93	1.90	21.96	(MICHEL)

Proteose-like substances have been found in blood-serum by several investigators, and NOLF [3] has shown that after the abundant introduction of proteoses into the intestine, they pass into the blood. BORCHARDT [4] has also been able to show that not only after the introduction of elastin-proteose *per os*, but also after feeding dogs with not overabundant quantities of elastin, a proteose, hemielastin, passes into the blood and indeed can be eliminated by the urine. The question whether the proteoses are normal constituents of the blood under ordinary conditions is still much disputed. The difficulty in deciding this question lies in

[1] Johansson, Zeitschr. f. physiol. Chem., **9**; K. Starke, Maly's Jahresber., **11**.

[2] See Hopkins and Pinkus, Journ. of Physiol., **23**; Krieger, Ueber die Darstellung krystallinscher tierischer Eiweissstoffe, Inaug.-Dissert. Strassburg, 1899.

[3] Bull. Acad. Roy. Belg., 1903 and 1904.

[4] Zeitschr. f. physiol. Chem., **51** and **57**.

the fact that in the removal of the proteins a small amount of proteose-like substance is formed from other proteins (namely from the globin of the blood pigment), and on the other hand the proteoses can be precipitated with the other bodies. The question as to the physiological occurrence of proteoses in the blood or plasma must be considered as still undecided.[1]

In close relation to the proteoses stands perhaps the above-mentioned seromucoid, which was discovered by ZANETTI and especially studied by BYWATERS. It is a glycoprotein which is soluble in water, and precipitated by alcohol. Seromucoid contains according to BYWATERS[2] 11.9 per cent N, 1.8 per cent S, and yields approximately 25 per cent glucosamine. The quantity in the blood is 0.2—0.9 p m.

The Blood-serum.

As above stated, the blood-serum is the clear liquid which is pressed out by the contraction of the blood-clot. It differs chiefly from the plasma in the absence of fibrinogen and in containing an abundance of fibrin ferment. Considered qualitatively, the blood-serum contains the same chief constituents as the blood-plasma.

Blood-serum is a sticky liquid which is more alkaline toward litmus than the plasma. The specific gravity in man is 1.027 to 1.032, average 1.028. The color is more or less yellow; in human blood-serum it is pale yellow with a shade toward green, and in horses it is often amber-yellow. The serum is ordinarily clear; after a meal it may be opalescent, cloudy, or milky white, according to the amount of fat contained in the food.

Besides the above-mentioned bodies, the following constituents are found in the blood-plasma or blood-serum:

Fat occurs from 1–7 p. m. in fasting animals. After partaking of food the amount is increased to a great extent. *Fatty acids*, or *soaps*, *glycerin* (NICLOUX, FR. TANGL, and ST. WEISER[3]) *lecithin* and *cholesterin* are also found. Cholesterin occurs, according to HÜRTHLE,[4] at least in part, as fatty-acid esters (*serolin* according to BOUDET). According to LETSCHE[5] free cholesterin probably also occurs in the serum.

Sugar seems to be a physiological constituent of the plasma and

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[1] See especially Abderhalden, Zeitschr. f. physiol. Chem., **51**, and Biochem. Zeitschr., **8** and **10**, and E. Freund, *ibid.*, **7** and **9**, which also contains the literature.

[2] Biochem. Zeitschr., **15**.

[3] Nicloux, Compt. rend. soc. biol., **55**; Tangl and St. Weiser, Pflüger's Arch., **115**

[4] Zeitschr. f. physiol. Chem., **21**, where Boudet is also cited. In regard to the quantity of these esters in bird-serum, see Brown, Amer. Journ. of Physiol., **2**.

[5] Zeitschr. f. physiol. Chem., **53**.

serum. According to the investigations of many workers [1] the sugar found is dextrose. STRAUSS [2] has also detected levulose in blood-serum and in transudates and exudates. The question as to the occurrence of other varieties of sugar, such as isomaltose (PAVY and SIAU) and pentose (LÉPINE and BOULUD [3]), in blood serum is still undecided. ASHER and ROSENFELD and MICHAELIS and RONA in a more conclusive manner, have shown that at least a considerable part of the sugar can be removed from the blood by dialysis, hence it must exist in solution in the free state. These observations do not exclude the possibility of the existence of another part of the sugar which is in combination with protein. LÉPINE and BOULUD [4] could only obtain a diffusion of the sugar by a short dialysis from serum 12 hours old, but not from perfectly fresh serum, an observation which somewhat diminishes the conclusiveness of MICHAELIS and RONA's experiment with 24-hour dialysis. A further testing of this question is therefore very desirable. Besides sugar, the blood-serum contains, as first shown by J. OTTO, another reducing non-fermentable substance whose quantity in rabbits' blood is about one-quarter of the total quantity of reducing substance (N. ANDERSSON). The statements of JACOBSEN, HENRIQUES, and BING,[5] that this substance is jecorin or lecithin sugar, do not have sufficient foundation, and the identity with jecorin becomes more striking as the existence of a jecorin is on the whole doubtful. The nature of another carbohydrate in the blood, which is neither dextrorotatory nor reducing, and which has been called *virtual sugar* by its discoverers, LÉPINE and BOULUD,[6] is also undetermined. The virtual sugar is more abundant in the blood of the right ventricle than in the arterial blood, and this in turn is richer than venous blood. In the passage of the blood through the lungs the virtual sugar is converted into ordinary sugar; this may also occur in the capillaries of the greater circulatory system.

Conjugated glucuronic acids, which probably originate from the form-elements, have been shown to occur in blood by the researches of

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[1] See v. Mering, Arch. f. (Anat. u.) Physiol., 1877 (this article contains numerous references); Seegen, Pflüger's Arch., **40**; Miura, Zeitschr. f. Biologie, **32**.

[2] Fortschritte d. Mediz., 1902.

[3] Pavy and Siau, Journ. of Physiol., **26**; Lépine and Boulud, Compt. rend., **133**, **135**, and **136**.

[4] Rosenfeld, Centralbl. f. Physiol., **19**, p. 449; Lépine and Boulud, Compt. rend., **143**; Asher, Biochem. Zeitschr., **3**; Michaelis and Rona, *ibid.*, **14**.

[5] Otto, Pflüger's Arch., **35** (a good review of the older literature on sugar in the blood); N. Andersson, Biochem. Zeitschr., **12**; Jacobsen, Centralbl. f. Physiol., **6**, 368; Henriques, Zeitschr. f. physiol. Chem., **23**; Bing, Skand. Arch. f. Physiol., **9**; see also P. Mayer, Biochem. Zeitschr., **1** and **4** on jecorin and blood sugar.

[6] Compt. rend., **137**, **144**, **147**.

P. Mayer, Lépine and Boulud[1]. The last two investigators find two definite glucuronic acids in the blood, both of which are levorotatory. One reduces Fehling's solution even at a temperature below 100°, while the other reduces it at above 100°. Such large amounts of the first acid often occur in the blood of dogs that the optical activity of the glucuronic acid counteracts that of the glucose. The second acid also occurs in larger quantities as compared with the sugar.

Bernard[2] has shown that the quantity of sugar in the blood diminishes more or less rapidly on leaving the veins. Lépine, associated with Barral, has specially studied this decrease in the quantity of sugar, and calls it *glycolysis*. Lépine and Barral, as well as Arthus, have shown that this glycolysis takes place in the complete absence of micro-organisms. It seems to be due to a soluble *glycolytic enzyme* whose activity is destroyed by heating to 54° C. This enzyme is derived, according to the above investigators, from the leucocytes and, according to Arthus as well as to Doyon and Morel[3] it occurs only in the serum but not in the plasma. According to Lépine,[4] it has some connection with the pancreas. The glycolysis is, according to Röhmann and Spitzer and Sieber,[5] an oxidation which is produced, according to the two last-mentioned investigators, by an oxidation ferment. There is still some doubt whether this is a physiological process or not.[6]

The blood-plasma and the serum, as well as the lymph, also contain *enzymes* of various kinds. According to Röhmann, Bial, Hamburger,[7] and others, *diastases*, which convert starch and glycogen into maltose or isomaltose, as well as a *maltase*, are found in the blood. Hanriot has detected in the serum a *lipase* which decomposes butyrin, and which, according to him, decomposes neutral fats and other esters. The occur-

[1] Mayer, Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. rend., **133**, **135**, **136**, **138**, **141**, and Journ. de Physiol., **7** (cited from Biochem. Centralbl., **4**, p. 421).

[2] Leçons sur le diabète, Paris, 1877.

[3] Arthus, Arch. de Physiol. (5), **3**; Doyon and Morel, Compt. rend. soc. biol., **55**.

[4] In regard to the numerous memoirs of Lépine and Lépine and Barral, see Lyon médical., **62** and **63**; Compt. rendus, **110**, **112**, **113**, **120**, and **139**; Lépine, Le ferment glycolytique et la pathogénie du diabète (Paris, 1891), and Revue analytique et critique des travaux, etc., in Arch. de méd. expér. (Paris, 1892); Revue de médecine, 1895; Arthus, Arch. de Physiol. (5), **3**, **4**; Nasse and Framm, Pflüger's Arch., **63**, Paderi, Maly's Jahresber., **26**; see also Cremer, Physiologie des Glykogens in Ergebnisse d. Physiol., **1**, Abt. 1.

[5] Röhmann and Spitzer, Ber. d. d. chem. Gesellsch., **28**; Spitzer, Pflüger's Arch., **60** and **67**; Sieber, Zeitschr. f. physiol. Chem., **39** and **44**.

[6] See Arthus, l. c.; Colenbrander, Maly's Jahresber., **22**; Rywosch, Centralbl. f. Physiol., **11**, 495.

[7] Röhmann; Röhmann and Hamburger, Ber. d. deutsch. chem. Gesellsch., **25** and **27**; Pflüger's Arch., **52** and **60**; Bial, Ueber das diast. Ferm., etc., Inaug.-Diss. Breslau, 1892 (older literature). See also Pflüger's Arch., **52**, **54**, and **55**

rence of a *butyrinase* is generally admitted, while the property of this lipase of splitting olein and other neutral fats is not generally acknowledged (ARTHUS, DOYON and MOREL [1]). This lipolytic property, if it exists to the extent that HANRIOT ascribes to it, must not be confounded with the transformation of fat into unknown substances soluble in water, a phenomenon first observed by CONNSTEIN and MICHAELIS and further studied by WEIGERT. The occurrence of such a body is positively denied by G. MANSFELD.[2]

Besides the above-mentioned enzymes and thrombin, several other enzymes have been found in the blood-serum, namely, *oxidases, catalases, proteolytic* enzymes, among which we must mention the *polypeptide-splitting* enzymes studied by ABDERHALDEN and collaborators,[3] also *rennin* and several *antienzymes*. We cannot enter into the discussion of these, nor of the many not chemically characterized bodies which have been called *toxines* and *antitoxines, immune bodies, alexines, hæmolysines, cytotoxines*, etc. It is also not within the scope of this book to discuss the *precipitines* which can be used as a biological reagent on account of their action upon various proteins. It may be sufficient to state that the works of BORDET, EHRLICH, WASSERMANN, SCHÜTZE, UHLENHAUT,[4] and others have shown that the repeated injection into an animal of a foreign protein body or of blood of a different species of animal so changes the blood of this animal that it acquires precipitating properties toward the injected protein or blood. In this manner we obtain a biological reagent for various proteins and for blood of different animals. This last behavior has become of great forensic importance, due to the work of UHLENHAUT. The various enzymes and antienzymes, toxines and and antitoxines, precipitines, etc., are as a rule precipitated with the globulin, but differ among each other in that some are carried down by the euglobulin, while the others are carried down by the pseudoglobulin fraction.

The non-protein organic constituents of the serum have been especially carefully studied by E. LETSCHE [5] and he has found, besides the previously known bodies, that the serum contains several acids, among which there are two nitrogenous acids whose nature has not been studied. These, including other nitrogenous substances found by him, represent a part

[1] Hanriot, Compt. rend. soc. biol., **48** and **54**; Compt. rend., **123** and **132**; Arthus, Journ. de Physiol. et de Pathol., **4**; Doyon and Morel, Compt. rend. soc. biol., **54**; Achard and Clerç (Lipase in Disease), Compt. rend., **129**, and Arch. d. med. expér., **14**.

[2] Connstein and Michaelis, Pflüger's Arch., **65** and **69**; Weigert, *ibid.*, **82**; Mansfeld, Centralbl. f. Physiol., **21**.

[3] Zeitschr. f. physiol. Chem., **51**, **53**, **55**.

[4] The literature on this subject may be found in bacteriological journals and works. See also L. Michaelis, Biohcem. Centralbl., **3**, p. 693.

[5] Zeitschr. f. physiol. Chem., **53**.

of the so-called *rest nitrogen*, i.e., that nitrogen which remains in the serum after the complete removal of the coagulable proteins. As representatives of the bodies occurring as rest nitrogen in the serum we must in the first place mention *urea*, also *creatine*, *carbamic acid*, *ammonia*, *hippuric acid*, *phosphocarnic acid* (PANELLA [1]), traces of *indol* (HERVIEUX [2]), perhaps also *uric acid* found by ABELES [3] in human blood, while LETSCHE could not find any in horse-blood. LETSCHE could not find any mon-amino- or diamino-acids and purine bases which, like *lysine* (NEUBERG and RICHTER [4]), *leucine*, *tyrosine* and *bile-acids* are found in blood serum under pathological conditions.

According to BROWINSKI [5] proteic acids (see Chapter XV) occur in the serum. As above stated, the occurrence of proteoses is disputed. We have several investigations on the occurrence of amino-acids (v. BERGMANN, HOWELL, LETSCHE and others [6]) which make the occurrence of these very probable, and recently BINGEL [7] has been able to show the presence of glycocoll in normal ox-blood. That the quantity of rest nitrogen is larger during digestion than in starvation requires further confirmation (V. BERGMANN and LANGSTEIN, HOHLWEG and H. MEYER [8]).

The *pigments* of the blood-serum are very little known. Besides other pigments horse-serum contains, as first shown by HAMMARSTEN, bilirubin, which according to RANC is the only pigment of the serum of this animal. This pigment occurs according to BIFFI and GALLI in especially large quantities in the blood of new-born.[9] The yellow coloring-matter of the serum seems to belong to the group of *luteins*, which are often called *lipochromes* or fat-coloring matters. From ox-serum KRUKENBERG [10] was able to isolate with amyl alcohol a so-called lipochrome whose solution shows two absorption-bands, of which one encloses the line *F* and the other lies between *F* and *G*.

The *mineral bodies* in serum and plasma are qualitatively, but not quantitatively, the same. A part of the calcium, magnesium, and phosphoric acid is removed on the coagulation of the fibrin. By means of

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[1] Panella, cited in Virchow's Jahresb., 1902, 150.

[2] Compt. rend. soc. biol., **56**.

[3] Wien. med. Jahrb., 1887.

[4] Deutsch. med. Wochenschr., 1904.

[5] Zeitschr. f. physiol. Chem., **54** and **58**.

[6] v. Bergmann, Hofmeister's Beiträge, **6**; Howell, Amer. Journ. of Physiol., **17**; Letsche, l. c.

[7] Zeitschr. f. physiol. Chem., **57**.

[8] v. Bergmann and Langstein, Hofmeister's Beiträge, **6**; Hohlweg and Meyer, *ibid.*, **11**.

[9] Hammarsten, see Maly's Jahresb., 8 (1878); Ranc, Compt. rend. soc. biol., **62**; Biffi and Galli, Journ. de Physiol. et Path., **9** (1907).

[10] Sitz. Ber. d. Jen. Gesellsch. f. Med., 1885.

dialysis, the presence of sodium chloride, which forms the chief mass or 60–70 per cent of the total mineral bodies, lime-salts, sodium carbonate, and traces of sulphuric and phosphoric acids and of potassium, may be directly shown in the serum.[1] Traces of silicic acid, fluorine, copper, iron, manganese, and ammonia are claimed to have been found in the serum. As in most animal fluids, the chlorine and sodium are in the blood-serum in excess of the phosphoric acid and potassium (the occurrence of which in the serum is even doubted). The acids present in the ash are not sufficient to saturate the bases found, a condition which shows that a part of the bases is combined with organic substances, perhaps proteins. This also coincides with the fact that the great part of the alkalies does not exist in the serum as diffusible alkali compounds, carbonate and phosphate, but as non-diffusible compounds, protein combinations. According to HAMBURGER[2] 37 per cent of the alkali of the serum from horse-blood was diffusible and 63 per cent non-diffusible.

Iodine, which seems to be habitually found, is also considered as a mineral constituent of the plasma or serum (GLEY and BOURCET), while arsenic, although not found in all blood, occurs in human blood (GAUTIER, BOURCET[3]). Iodine occurs to a greater extent in menstrual blood than in other blood and does not exist as a salt, but as an organic compound (BOURCET).

The *gases* of the blood-serum, which consist chiefly of carbon dioxide with only a little nitrogen and oxygen, will be described when treating of the gases of the blood.

We have only a few analyses of blood-plasma. As an example the results of the analyses of the blood-plasma of the horse will be given below. The analysis No. 1 was made by HOPPE-SEYLER.[4] No. 2 is the average of the results of three analyses made by HAMMARSTEN. The figures are given for 1000 parts of the plasma.

	No. 1.	No. 2.
Water	908.4	917.6
Solids	91.6	82.4
Total proteins	77.6	69.5
Fibrin	10.1	6.5
Globulin	.....	38.4
Seralbumin	.....	24.6
Fat	1.2	12.9
Extractive substances	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

[1] See Gürber, Verhandl. d. phys.-med. Gesellsch. zu Würzburg, **23**.

[2] In regard to method, see Arch. f. (Anat. u.) Physiol., 1898.

[3] Gley and Bourcet, Compt. rend., **130**; Bourcet, *ibid.*, **131**; Gautier, *ibid.*, **131**.

[4] Cit. from v. Gorup-Besanez's Lehrbuch der physiol. Chem., 4. Aufl., 346.

LEWINSKY[1] has determined the total proteins and the individual proteins in the blood-plasma of man and animals with the following results:

	Total Protein.	Albumin.	Globulin.	Fibrinogen.
Man	72.6	40.1	28.3	4.2
Dog	60.3	31.7	22.6	6.0
Sheep	72.9	38.3	30.0	4.6
Horse	80.4	28.0	47.9	4.5
Pig	80.5	44.2	29.8	6.5

ABDERHALDEN has made complete analyses of the blood-serum of several domestic animals. From these analyses, as well as from those made by HAMMARSTEN of the serum from human, horse, and ox-blood, it follows that the amount of solids ordinarily varies between 70–97 p. m. The chief mass of the solids consists of proteins, about 55–84 p. m. In hens HAMMARSTEN found much lower values, namely, 54 p. m. solids, with only 39.5 p. m. protein, and HALLIBURTON found only 25.4 p. m. protein in frog's blood. The relation between globulin and seralbumin is, as shown by the analyses of HAMMARSTEN, HALLIBURTON, and RUBBRECHT,[2] very different for various animals, but may also vary considerably in the same species of animal. In human blood-serum HAMMARSTEN found more seralbumin than globulin, and the relation of serglobulin to seralbumin was as 1:1.5. LEWINSKY found the relaship in man greater than 1, indeed 1:1.39–2.13. In regard to the quantity of the remaining organic constituents of the serum we refer the reader to ABDERHALDEN'S complete analyses.

In starvation it seems, as first found by BURCKHARDT and recently substantiated by GITHENS,[3] that the quantity of globulins relative to that of albumin is increased. A change in the relation with a decrease in the albumin and an increase in the globulin may also occur in animals which have been made sick or in part immune by inoculation with pathogenic microorganisms (LANGSTEIN and MAYER[4]). The total protein content is raised in nearly all cases. The amount of fibrinogen in the plasma is especially increased by pneumococci, streptococci, and pus-staphylococci (P. MÜLLER[5]).

The quantity of mineral bodies in the serum has been determined by many investigators. The conclusion drawn from the analyses is that there

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[1] Pflüger's Arch., **100**.

[3] Abderhalden, Zeitschr. f. physiol. Chem., **25**; Hammarsten, Pflüger's Arch., **17**; Halliburton, Journ. of Physiol., **7**; Rubbrecht, Travaux du laboratoire de l'institut de physiologie de Liège, **5**, 1896.

[3] Burckhardt, Arch. f. exp. Path. u. Pharm., **16**; Githens, Hofmeister's Beiträge, **5**; see also Morawitz, *ibid.*, **7**, and Inagaki, Zeitschr. f. Biol., **49**.

[4] Hofmeister's Beiträge, **5**.

[5] *Ibid.*, **6**.

exists a rather close correspondence between human and animal blood-serum, and it is therefore sufficient to give here the analysis of C. SCHMIDT [1] of (1) human blood, and BUNGE and ABDERHALDEN'S analyses (2) of serum of ox, bull, sheep, goat, pig, rabbit, dog, and cat. The results correspond to 1000 parts by weight of the serum.

	1	2
K_2O	0.387–0.401	0.226–0.270
Na_2O	4.290–4.290	4.251–4.442
Cl	3.565–3.659	3.627–4.170
CaO	0.155–0.155	0.119–0.131
MgO	0.101	0.040–0.046
P_2O_5 (inorg.)		0.052–0.085

Even if we bear in mind that certain bodies, such as carbon dioxide, are driven off during incineration, and that other bodies, such as sulphuric acid and phosphoric acid, are formed from sulphurized and phosphorized organic substances, still quantitative analyses like the above are not sufficient for the scientific demands of to-day. They do not show the true composition, and especially do not give an explanation of the number of different ions present in the serum or in other fluids, a question which is of the greatest physiological importance. An answer to these questions is obtainable only by physico-chemical investigations, which have thus far been used chiefly in determining the molecular concentration, the amount of electrolytes and non-electrolytes, and the degree of dissociation.

The average depression of the freezing-point of mammalian blood corresponds, as already given in Chapter II, closely to a 9 p. m. ($\Delta = -0.551$–$0.561°$) solution of common salt, and at the present time such a solution is considered as a physiological salt solution for man and other mammalia. In lower animals and fish the conditions are otherwise, as shown in the above-mentioned chapter.

There are recorded a great number of investigations on the changes in the osmotic pressure or the molecular concentration of the blood-serum under various physiological conditions as well as in disease, but still it is no doubt too early to draw any definite conclusions from these observations.

The degree of dissociation (see Chapter II) of sera has been determined by several investigators, and according to HAMBURGER [2] it lies between 0.65 and 0.82. The molecular concentration, which represents the total number of molecules and ions per liter is, according to BUGARSKY and TANGL, on an average of about 0.320 mol. per liter. They also

[1] Cit. from Hoppe-Seyler, Physiol. Chem., 1881, p. 439.

[2] Osmotisher Druck und Ionenlehre, Wiesbaden, 1902–1904, where the literature on the physical chemistry of the blood can be found.

found that about three-fourths of the total number of dissolved molecules in blood-serum were electrolytes, although the serum contained about 70–80 p. m. protein and 10 p. m. inorganic bodies, and also that three-fourths of the quantity of electrolytes consisted of NaCl.

In the determination of the alkalinity of blood and blood-serum, up to the present time we have estimated the amount of alkali by titration with an acid. We cannot dispense with such determinations, although they do not yield any information as to the true alkalinity, apart from the fact that the results are dependent upon the indicator used, because we understand as true alkalinity the concentration of the hydroxyl ions. The Na_2CO_3 is in aqueous solution more or less dissociated into $2Na^+$ and $CO_3^=$, depending upon the dilution. The $CO_3^=$ ions combine partly with the H^+ ions of the dissociated water, forming HCO_3^-, and the corresponding HO^- ions produce the alkaline reaction. If now by the addition of a little acid, a few of the HO^- ions are removed, then the equilibrium is disturbed, a new quantity of Na_2CO_3 is dissociated, and this process is repeated every time a new quantity of acid is added until all the carbonate is dissociated. The dissociation of the carbonate existing in the original concentration, upon which the number of HO^- ions is dependent, cannot therefore be determined by titration. For these reasons Höber has worked out a physical-chemical method of determining alkalinity, based upon Nernst's theory of liquid chains. This method was used later by Farkas, Fränckel, and Höber after a few changes. The investigations of these last-mentioned experimenters show that the concentration of the hydroxyl ions in blood-serum and blood is nearly the same as in distilled water, and that these fluids are nearly neutral in behavior, which is accounted for by the presence of carbonic acid. Friedenthal,[1] by testing serum with phenolphthalein, arrived at similar results.

II. THE FORM-ELEMENTS OF THE BLOOD.

The Red Blood-corpuscles.

The blood-corpuscles are round, biconcave disks without membrane and nucleus in man and mammalia (with the exception of the llama, the camel, and their congeners). In the latter animals, as also in birds, amphibia, and fish (with the exception of the Cyclostoma), the corpuscles have in general a nucleus, are biconvex and more or less ellipitical. The size varies in different animals. In man they have an average diameter of 7 to 8 μ (μ=0.001 mm.) and a maximum thickness of 1.9 μ. They are heavier than the blood-plasma or serum, and therefore sink in these

[1] Höber, Pflüger's Arch., **81** and **99**; Farkas, see Biochem. Centralbl., **1**, 626; Fränckel, Pflüger's Arch., **96**; Friedenthal, Zeitschr. f. allg. Physiol., **1** and **4**.

liquids. In the discharged blood they may sometimes lie with their flat surfaces together, forming a cylinder like a roll of coin (rouleaux). The reason for the phenomenon, which is considered as an agglutination, has not been sufficiently studied, but as it may be observed in defibrinated blood it seems probable that the formation of fibrin has nothing to do with it.

The number of red blood-corpuscles is different in the blood of various animals. In the blood of man there are generally 5 million red corpuscles in 1 c.mm., and in woman 4 to 4.5 million.

The blood-corpuscles consist essentially of two chief constituents, the stroma, which forms the real protoplasm, and the intraglobular contents, whose chief constituent is hæmoglobin. We cannot state anything positive for the present in regard to a more detailed arrangement, and the views on this subject are somewhat divergent. The two following views are more or less related to each other. According to one view the blood-corpuscles consist of a membrane which encloses a hæmoglobin solution, while the other view considers the stroma as a protoplasmic structure soaked with hæmoglobin. This latter view is in accord with the assumption as to an outside boundary-layer.

Thus according to HAMBURGER the stroma forms a protoplasmic net in whose meshes there exists a red fluid or semi-fluid mass which consists in great measure of hæmoglobin. This mass represents the water-attracting force of the blood-corpuscles, and besides this it is also considered that the outer protoplasmic boundary is semi-permeable, i.e., permeable to water but not permeable to certain crystalloids. The researches of KÖPPE, ALBRECHT, PASCUCCI, RYWOSCH,[1] and others indicate the presence of a special envelope or boundary-layer, and there is no doubt that the outer layer contains so-called lipoids, such as cholesterin, lecithin, and similar bodies.

The red blood-corpuscles retain their volume in a salt solution which has the same osmotic pressure as the serum of the same blood, although they may change their form in such solutions, becoming more spherical, and may also undergo a chemical change. Such a salt solution is *isotonic* with the blood-serum, and its concentration for a NaCl solution is approximately 9 p. m. for human and mammalian blood. A solution of greater concentration, a *hyperisotonic* solution, abstracts water from the blood-corpuscles until osmotic equilibrium is established, hence the corpuscles shrink and their volumes become smaller. In solutions of less concentration, *hypisotonic* solutions, the corpuscles swell up, due to the taking up of water, and this swelling may be so great, as on diluting

[1] See Hamburger, Osmotischer Druck und Ionenlehre, 1902; Köppe, Pflüger's Arch., **99** and **107**; Albrecht, Centralbl. f. Physiol., **19**; Pascucci, Hofmeister's Beiträge, **6**; Rywosch, Centralbl. f. Physiol., **19**.

the blood with water, that the hæmoglobin is separated from the stroma and passes into the watery solution. This process is called *hæmolysis*, (see Chapter II).

A hæmolysis may also be brought about by alternately freezing and thawing the blood, as well as by the action of various chemical substances, which act as protoplasmic poisons. These bodies are ether, chloroform alkalies, bile-acids, solanin, saponin, and also the saponin substances, which have a very strong hæmolytic action. Of special interest in this regard are the hæmolysines, which act like toxines. These hæmolysines may be metabolic products of bacteria and may be formed by higher plants and by animals, such as snakes, toads, bees, spiders, and others. Finally, the hæmolysines or globulicidal bodies, occurring normally in blood-sera or produced in the immunization of the blood, also belong here.

It seems that hæmolysis is brought about in various cases in different ways. In the hæmolysis by means of water we are probably dealing with a destruction or rupture of the boundary-layer, while such bodies as ether, chloroform, alkalies, bile-acids, and saponin substances, which dissolve lipoids or form combinations therewith, in this way cause the passage of the hæmoglobin to the outside (Köppe, Ransom and Kobert, Peskind, Pascucci). The action of other hæmolysines, such as snake-venom and tetanotoxine, seems to be an action connected with the lecithin (Keyes, Pascucci [1]).

When the hæmoglobin is separated from the so-called stroma by a sufficiently strong dilution with water the stroma is found in the solution in a swollen condition. By the action of carbon dioxide, by the careful addition of acids, acid salts, tincture of iodine, or certain other bodies, this residue, rich in proteins, condenses, and in many cases the form of the blood-corpuscles may be again obtained. This residue, the so-called ghosts or stromata of the blood-corpuscles, can also be directly colored in dilute blood by methyl violet and in this way detected (Köppe), and attempts have been made to isolate it for chemical investigation. In the following pages we mean by the name stroma only that residue that remains after the removal of hæmoglobin and other bodies soluble in water.

To isolate the stromata from the blood-corpuscles, they are washed first by diluting the blood with 10–20 vols. of a 1–2 per cent common salt solution and then separating the mixture by centrifugal force or by allowing it to stand at a low temperature. This is repeated a few times until the blood-corpuscles are freed from serum. These purified blood-corpuscles are, according to Wooldridge, mixed with 5–6 vols. of water and then a little ether is added until complete solution is obtained. The leucocytes gradually settle to the bottom, a movement which may be

[1] Köppe, l. c.; Peskind, Amer. Journ. of Physiol., **12**; Ransom and Kobert, cited by Pascucci, Hofmeister's Beiträge, **6**; Keyes, Zeitschr. f. physiol. Chem., **41**, and Berl. klin. Wochenschr., 1904.

accelerated by centrifugal force, and the liquid which separates therefrom is very carefully treated with a 1 per cent solution of $KHSO_4$ until it is about as dense as the original blood. The separated stromata are collected on a filter and quickly washed. PASCUCCI,[1] on the contrary, treats the mass of corpuscles with 15–20 vols. of a one-fifth saturated ammonium-sulphate solution, allows the corpuscles to settle, siphons off the fluid, repeatedly centrifuges, allows the residue to dry quickly (on porcelain plates) at the ordinary temperature, and then washes with water until the blood-pigments and the other soluble bodies are dissolved out.

WOOLDRIDGE found as constituents of the stromata *lecithin, cholesterin, nucleoalbumin,* and a *globulin* which, according to HALLIBURTON, is probably a nucleoproteid which he calls *cell-globulin.* No nuclein substances or seralbumin or proteoses could be detected by HALLIBURTON and FRIEND. According to PASCUCCI, the stromata (from horse-blood) consists of one-third cholesterin and lecithin (besides a little cerebroside), and two-thirds protein substances and mineral bodies. The nucleated red blood-corpuscles of the bird contain, according to PLÓSZ and HOPPE-SEYLER,[2] a protein (nucleoprotein) which swells to a slimy mass in a 10 per cent common salt solution, and which seems to be closely related to the hyaline substance (*hyaline substance* of ROVIDA, see page 295) occurring in the lymph-cells. In the mass extracted by alcohol from the blood-corpuscles of the hen, ACKERMANN found 3.93 per cent phosphorus and 17.2 per cent nitrogen, which on calculation gave 42.10 per cent nucleic acid and 57.82 per cent histone. PIETTRE and VILA[3] found in the stromata 0.3 per cent phosphorus in the horse and 2.3–2.6 per cent in birds (ducks and hens) calculated on the ash-free substance. They found the quantity of nitrogen to be 11.7 and 13.21 per cent for the horse and dog respectively. The non-nucleated red blood-corpuscles are, as a rule, very poor in protein, but are rich in hæmoglobin; the nucleated corpuscles are richer in protein and poorer in hæmoglobin than the non-nucleated. Several enzymes probably also occur as constituents of the stromata and among these occurs the proteolytic enzyme studied by ABDERHALDEN and collaborators.[4] It is difficult to decide in many cases whether the enzymes found in the blood belong to the fluid or to the various kinds of form-elements.

A gelatinous, fibrin-like protein body may be obtained from the red blood-corpuscles under certain circumstances. This fibrin-like mass has been observed on freezing and then thawing the sediment of the blood-corpuscles, or on discharging the spark from a large Leyden jar through

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[1] Hofmeister's Beiträge, **6**.

[2] Wooldridge, Arch. f. (Anat. u.) Physiol., 1881, 387; Halliburton and Friend, Journal of Physiol., **10**; Halliburton, *ibid.*, **18**; Plósz, Hoppe-Seyler's Med. chem. Untersuch., 510.

[3] Ackermann, Zeitschr. f. physiol. Chem., **43**; Piettre and Vila, Compt. Rend., **143**.

[4] Zeitschr. f. physiol. Chem., **51**, **53** and **55**.

the blood, or on dissolving the blood-corpuscles of one kind of animal in the serum of another (LANDOIS, *stroma-fibrin*); i.e., in the so-called *hæmagglutination*, a clumping of the red blood-corpuscles into clusters takes place. This agglutination can be brought about by bodies similar to the hæmolysines and also by serum constituents produced normally or by immunization. It has not been shown that a fibrin formation from the stroma takes place. Fibrinogen has only been detected in the red corpuscles of frog's blood (ALEX. SCHMIDT and SEMMER [1]).

Closely related to the anatomical and chemical structure of the erythrocytes is the question which is important, for the metabolism in the blood, as to the permeability of the erythrocytes, that is, their power of taking up substances of different kinds. This question as well as the permeability of the blood-corpuscles for anions under the influence of carbon dioxide has been discussed in a previous chapter (II, page 33).

The *mineral bodies* of the red corpuscles will be treated in connection with their quantitative constitution.

The constituent of the blood-corpuscles existing in greatest quantity is the red pigment hæmoglobin.

Blood-pigments.

According to HOPPE-SEYLER the coloring-matter of the red blood-corpuscles is not in a free state, but combined with some other substance. The crystalline coloring-matter, the hæmoglobin or oxyhæmoglobin, which may be isolated from the blood, is considered, according to HOPPE-SEYLER, as a cleavage product of this compound, and it acts in many ways unlike the questionable compound itself. This compound is insoluble in water and uncrystallizable. It strongly decomposes hydrogen peroxide without being oxidized itself; it shows a greater resistance to certain chemical reagents (as potassium ferricyanide) than the free coloring-matter; and, lastly, it gives off its loosely combined oxygen much more easily in vacuum than the free pigment. To distinguish between the cleavage products, the hæmoglobin and the oxyhæmoglobin, HOPPE-SEYLER calls the compound of the blood-coloring matter of the venous blood-corpuscles *phlebin*, and that of the arterial *arterin*. Other investigators, such as H. U. KOBERT and BOHR,[2] the latter calling the pigment

[1] Landois, Centralbl. f. d. med. Wissensch., 1874, 421; Schmidt, Pflüger's Arch., **11**, 550–559.

[2] Hoppe-Seyler, Zeitschr. f. physiol. Chem., **13**, 479; H. U. Kobert, Das Wirbeltierblut in mikro-kristallogr. Hinsicht, Stuttgart, 1901; Bohr, Cenrtalbl. f. Physiol., **17**, p. 688.

of the blood-corpuscles *hæmochrom*, are of a similar opinion. Since the above-mentioned combinations of the blood-coloring matters with other bodies, for example (if they really do exist) with lecithin, have not been closely studied, the following statements will apply only to the free pigment, the hæmoglobin.

The color of the blood depends in part on *hæmoglobin* and in part on a molecular combination of this substance with oxygen, the *oxyhæmoglobin*. We find in blood after asphyxiation almost exclusively hæmoglobin, in arterial blood disproportionately large amounts of oxyhæmoglobin, and in venous blood a mixture of both. Blood-coloring matters are also found in striated as well as in certain smooth muscles, and lastly in solution in different invertebrates. The quantity of hæmoglobin in human blood may indeed be somewhat variable under different circumstances, but amounts to about 14 per cent on an average, or 8.5 grams for each kilo of the weight of the body.

Hæmoglobin belongs to the group of compound proteins, and yields as cleavage products, besides very small amounts of volatile fatty acids and other bodies, chiefly a protein *globin*, and a coloring-matter, *hæmochromogen* (about 4 per cent), containing iron, which in the presence of oxygen is easily oxidized into *hæmatin*.

As first shown by Schunck and Marchlewski, and especially by the work of the latter, a close relation exists between chlorophyll and the blood-pigment, because a derivative of the first, phylloporphyrin, stands very close in certain respects to a derivative of the blood-pigment hæmatoporphyrin. By the investigations of Nencki in conjunction with Marchlewski and Zaleski,[1] it was shown that hæmopyrol could be prepared from the derivatives of both the leaf-pigment and the blood-pigments by reduction. The fact that chlorophyll and blood-pigments are closely related and are constructed from the same mother-substance, is of the greatest biological importance.

The hæmoglobin prepared from different kinds of blood has not exactly the same composition, which seems to indicate the presence of different hæmoglobins. The analyses by different investigators of the hæmoglobin from the same kind of blood do not always agree with one another, which probably depends upon the somewhat varying methods of preparation. The following analyses are given as examples of the constitution of different hæmoglobins:

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[1] Schunck and Marchlewski, Annal. d. Chem. u. Pharm., **278**, 284, 288, 290; Nencki. Ber. d. deutsch. chem. Gesellsch., **29**; Marchlewski and Nencki, Ber. d. d. chem, Gesellsch., **34**; Nencki and Zaleski, *ibid.*; Marchlewski, Chem. Centralbl., 1902, I, 1016; Zaleski, Zeitschr. f. physiol. Chem., **37**.

Hæmoglobin from the	C	H	N	S	Fe	O	P_2O_5	
Dog	53.85	7.32	16.17	0.390	0.430	21.84	....	(Hoppe-Seyler)
"	54.57	7.22	16.38	0.568	0.336	20.93	....	(Jaquet)
Horse	54.87	6.79	17.31	0.650	0.470	19.73	....	(Kossel)
"	51.15	6.76	17.94	0.390	0.335	23.43	....	(Zinoffsky)
Ox	54.66	7.25	17.70	0.447	0.400	19.543	....	(Hüfner)
Pig	54.17	7.38	16.23	0.660	0.430	21.360	....	(Otto)
"	54.71	7.38	17.43	0.479	0.399	19.602	....	(Hüfner)
Guinea-pig	54.12	7.36	16.78	0.580	0.480	20.680	....	(Hoppe-Seyler)
Squirrel	54.09	7.39	16.09	0.400	0.590	21.440	....	"
Goose	54.26	7.10	16.21	0.540	0.430	20.690	0.770	"
Hen	52.47	7.19	16.45	0.857	0.335	22.500	0.197	(Jaquet)

That the repeatedly observed quantity of phosphorus in the hæmoglobin of birds (Inoko and others) is due to a contamination has been proven by Abderhalden and Medigreceanu. In the hæmoglobin from the horse (Zinoffsky), the pig, and the ox (Hüfner) we have 1 atom of iron to 2 atoms of sulphur, while in the hæmoglobin from the dog (Jaquet) the relation is 1 to 3. From the data of the elementary analysis, as also from the amount of loosely combined oxygen, Hüfner[1] has calculated the molecular weight of dog-hæmoglobin as 14,129, and the formula $C_{636}H_{1025}N_{164}FeS_3O_{181}$. According to the more recent determinations of Hüfner and Jaquet,[2] ox-hæmoglobin contains an average of 0.336 per cent iron, from which a molecular weight of 16,669 may be calculated. Hüfner and Gansser[3] have attempted to learn the size of the molecular weight of hæmoglobin by means of osmotic pressure determinations, and they found the following approximate results: for horse hæmoglobin 15,115 and for ox-hæmoglobin 16,321. The hæmoglobin from various kinds of blood not only shows a diverse constitution, but also a different solubility and crystalline form, and a varying quantity of water of crystallization; hence we infer that there are several kinds of hæmoglobin. Bohr is a very zealous advocate of this supposition. He has been able to obtain hæmoglobins from dog- and horse-blood, by fractional crystallization, which had different powers of combining with oxygen and contained different quantities of iron. Hoppe-Seyler had already prepared two different forms of hæmoglobin crystals from horse-blood, and Bohr concludes from all these observations that the ordinary hæmoglobin consists of a mixture of different hæmoglobins. In opposition to this statement, Hüfner[4] has shown

[1] Hoppe-Seyler, Med. chem. Untersuch., 370; Jaquet, Zeitschr. f. physiol. Chem., **14**, 296; Kossel, *ibid.*, **2**, 150; Zinoffsky, *ibid.*, **10**; Hüfner, Beitr. z. Physiol., Festschr. f. C. Ludwig, 1887, 74–81, Journ. f. prakt. Chem. (N. F.), **22**; Otto, Zeitschr. f. physiol. Chem., **7**; Inoko, *ibid.*, **18**; Abderhalden and Medigreceanu, *ibid.*, **59**.

[2] Arch. f. (Anat. u.) Physiol., 1894.

[3] Arch. f. (Anat. u.) Physiol., 1907.

[4] Bohr, "Sur les combinaisons de l'hémoglobine avec l'oxygène," Extrait du Bulletin de l'Académie Royale Danoise des sciences, 1890; also Centralbl. f. Physiol., 1890, 249. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **2**; Hüfner, Arch. f. (Anat. u.) Physiol., 1894.

that only one hæmoglobin exists in ox-blood, and that this is probably true for the blood of many other animals.

Oxyhæmoglobin, which has also been called HÆMATOGLOBULIN or HÆMATOCRYSTALLIN, is a molecular combination of hæmoglobin and oxygen. For each molecule of hæmoglobin 1 molecule of oxygen is present, as shown by the investigations of HÜFNER as well as HÜFNER and GANSSER, and the amount of loosely combined oxygen which is united to 1 gram of hæmoglobin (of the ox) has been determined by HÜFNER [1] as 1.34 cc. (calculated at 0° C. and 760 mm. mercury).

According to BOHR, the facts are different. He differentiates between four oxyhæmoglobins, according to the quantity of oxygen which they absorb, namely α-, β-, γ- and δ-oxyhæmoglobin, all having the same absorption-spectrum, and 1 gram combining with respectively 0.4, 0.8, 1.7, and 2.7 cc. oxygen at the temperature of the room and with an oxygen pressure of 150 mm. mercury. The γ-oxyhæmoglobin is the ordinary one obtained by the customary method of preparation. BOHR designates as α-oxyhæmoglobin the crystalline powder obtained by drying γ-oxyhæmoglobin in the air. On dissolving α-oxyhæmoglobin in water it is converted into β-oxyhæmoglobin without decomposition, and the quantity of iron is increased. On keeping a solution of γ-oxyhæmoglobin in a sealed tube it is transformed into δ-oxyhæmoglobin, although the exact conditions under which this change takes place are not known. According to HÜFNER [2] these are nothing but mixtures of genuine and partly decomposed hæmoglobins.

The ability of hæmoglobin to take up oxygen seems to be a function of the iron it contains, and when this is calculated as about 0.33–0.40 per cent, then 1 atom of iron in the hæmoglobin corresponds to about 2 atoms or 1 molecule of oxygen. By increasing the partial pressure as well as by increasing the quantities of oxygen, the hæmoglobin in solution takes up more oxygen, until it is completely saturated, when 1 molecule of hæmoglobin is combined with 1 molecule of oxygen. Still this reaction is reversible according to the type $1(\mathrm{Hb}) + 1(\mathrm{O_2}) \rightleftarrows 1(\mathrm{OHb})$, and with diminished oxygen pressure a dissociation must take place, with the giving up of oxygen and a re-formation of hæmoglobin. The equilibrium between oxyhæmoglobin, hæmoglobin, and oxygen is determined according to the law of mass-action, and according to the investigations of HÜFNER it is possible to calculate the relationship between oxyhæmoglobin (OHb) and hæmoglobin (Hb), at every desired partial pressure of the oxygen, by a formula suggested by him. According to BOHR [3] this formula does not have sufficient basis and does not correspond to the facts. BOHR found, in opposition to HÜFNER'S statements, that with the same oxygen tension the absorption of oxygen by a hæmoglobin solution changes with the concentration, and that a dilute solution

[1] Arch. f. (Anat. u.) Physiol., 1901, Suppl.
[2] Arch. f. (Anat. u.) physiol., 1894.
[3] Bohr, Centralbl. f. Physiol., **17**, pp. 682 and 688.

combines with more oxygen, calculated per 1 gram hæmoglobin, than a concentrated solution. Bohr suggested another formula expressing the relationship between the oxygen absorption and the oxygen tension, based upon the assumption that, besides the dissociation of the oxygen-hæmoglobin compound, a dissociation of the hæmoglobin into a part containing iron and a part containing no iron also takes place. This formula, which in fact accords well with Bohr's findings, is nevertheless only true for a hæmoglobin solution and not for blood, as, according to Bohr, the blood-pigment in the blood-corpuscles (the hæmochrom) is changed on being converted into hæmoglobin. Henri [1] also finds that Hüfner's formula for the dissociation of oxyhæmoglobin is not useful.

The native pigment, the hæmochrom, combines, according to Bohr, in maximum with the same quantity of oxygen as the corresponding hæmoglobin, when the latter is prepared without the use of violent methods; still from this it does not follow that the oxygen combination in hæmochrom is identical with that in hæmoglobin. According to Bohr this is not the case, at least with diminished pressure, for with low oxygen tension more oxygen is taken up by the blood than by a corresponding hæmoglobin solution. The curve showing the oxygen absorption is lower in this case for a hæmoglobin solution than for blood. The reason for this lies, according to Bohr, in the fact that the tension curve is influenced by the form of union of the part of the hæmoglobin containing iron with the iron-free part, and that this union is changed because of changes in the iron-free part, as by the splitting off of lecithin, etc. The tension curve of the oxygen in the blood can, according to Bohr, be determined only by direct experiments on the blood itself and not by experiments upon hæmoglobin solutions.

The elucidation of these conditions is of the very greatest importance, as the dependence of the reaction between OHb, Hb, and O upon the law of mass-action is naturally of the very greatest moment for the taking up of oxygen in the lungs and the giving up of the same to the tissues. The dissociation of the oxyhæmoglobin makes it also possible to completely expel the oxygen from a hæmoglobin solution or from blood by means of a vacuum or by passing an indifferent gas through the blood.

Oxyhæmoglobin, which is generally considered as a weak acid, is dextrorotatory, according to Gamgee.[2] The specific rotation for light of medium wave-length of C is $(\alpha)C = \text{about } +10°$, which corresponds also for carbon-monoxide hæmoglobin. The hæmoglobin is also, like carbon-monoxide hæmoglobin (COHb) and methæmoglobin (MHb), diamagnetic, while the hæmatin, which is richer in iron, is strongly mag-

[1] Henri, Compt. rend. soc. biolog., 56.

[2] Hofmeister's Beiträge, 4.

netic (GAMGEE [1]). On passing an electric current through an oxyhæmoglobin solution, the pigment first separates unchanged at the anode in a colloidal but still soluble form, and is then gradually transferred to the cathode in the colloidal state (GAMGEE [2]). This transportation of the colloidal hæmoglobin may also be made to take place through an animal membrane or through parchment paper. According to GAMGEE, the hæmoglobin probably exists in such a colloidal condition in the blood-corpuscles.

Oxyhæmoglobin has been obtained in crystals from several varieties of blood. These crystals are blood-red, transparent, silky, and may be 2–3 mm. long. The oxyhæmoglobin from squirrel's blood crystallizes in six-sided plates of the hexagonal system; the other varieties of blood yield needles, prisms, tetrahedra, or plates which belong to the rhombic system.[3] The quantity of water of crystallization varies between 3–10 per cent for the different oxyhæmoglobins. When completely dried at a low temperature over sulphuric acid the crystals may be heated to 110–115° C. without decomposition. At higher temperatures, somewhat above 160° C., they decompose, giving an odor of burnt horn, and leave, after complete combustion, an ash consisting of oxide of iron. The oxyhæmoglobin crystals from difficultly crystallizable kinds of blood, for example from such as ox's, human, and pig's blood, are easily soluble in water. The oxyhæmoglobins from easily crystallizable blood, as from that of the horse, dog, squirrel, and guinea-pig, are soluble with difficulty in the order above given. The oxyhæmoglobin dissolves more easily in a very dilute solution of alkali carbonate than in pure water, and this solution may be kept. The presence of a little too much alkali causes the oxyhæmoglobin to quickly decompose. The crystals are insoluble in absolute alcohol without decolorization. According to NENCKI,[4] it is hereby converted into an isomeric or polymeric modification, called by him *parahæmoglobin*. Oxyhæmoglobin is insoluble in ether, chloroform, benzene, and carbon disulphide.

A solution of oxyhæmoglobin in water is precipitated by many metallic salts, but is not precipitated by sugar of lead or basic lead acetate. On heating the watery solution it decomposes at about 70° C., and splits off protein and hæmatin when sufficiently heated. It is also readily

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[1] Proceedings of Roy. Society, **68**.

[2] *Ibid.*, **70**.

[3] The observation of Uhlik (Pflüger's Arch., **104**) that the hæmoglobin from horse-blood can also crystallize in hexagonal six-sided plates seems to be due to the fact that he had hæmoglobin and not oxyhæmoglobin.

[4] Nencki and Sieber, Ber. d. d. chem. Gesellsch., **18**. According to Krüger (see Biochem. Centralbl., I, 40, 463) hæmoglobin is somewhat changed by alcohol as well as by chloroform.

decomposed by acids, alkalies, and many metallic salts. It gives the ordinary reactions for proteins with those protein reagents which first decompose the oxyhæmoglobin with the splitting off of protein. Oxyhæmoglobin, like the other blood-pigments, has a direct oxidizing action upon tincture of guaiacum. It has, on the other hand, like all blood-pigments containing iron, the property of an "ozone transmitter" in that it turns tincture of guaiacum blue in the presence of reagents containing peroxide, such as old turpentine.

A sufficiently dilute solution of oxyhæmoglobin or arterial blood shows a spectrum with two absorption-bands between the FRAUNHOFER lines *D* and *E* (spectrum plate 1). The one band, α, which is narrower but darker and sharper, lies on the line *D*; the other, broader, less defined and less dark band, β, lies at *E*. The middle of the first band corresponds to a wave-length $\lambda=579$ and the second $\lambda=542$. On dilution the band β first disappears. By increased concentration of the solution the two bands become broader, the space between them smaller or entirely obliterated, and at the same time the blue and violet part of the spectrum is darkened. Besides these two bands we can also observe by the aid of special appliances (L. LEWIN, MIETHE, and STENGER) the band described by GAMGEE in the ultra-violet portion. This violet band, $\lambda=415$, is of importance in the detection of very small quantities of blood. While the two oxyhæmoglobin bands are still detectable in a dilution of 1:14,700 the violet band may be seen, according to LEWIN, MIETHE and STENGER [1] in a dilution of 1:40,000.

The observation of PIETTRE and VILA that so-called laky blood and oxyhæmoglobin solutions in thick layers also show a third band in the red ($\lambda=634$) depends in all probability, as also claimed by VILLE and DERRIEN, upon a partial formation of methæmoglobin which according to ARON [2] exists preformed in all blood.

A great many methods have been proposed for the preparation of oxyhæmoglobin crystals, but in their chief features they all agree with the following one suggested by HOPPE-SEYLER: The washed blood-corpuscles (best those from the dog or the horse) are stirred with 2 vols. water and then shaken with ether. After decanting the ether and allowing the ether which is retained by the blood solution to evaporate in an open dish in the air, cool the filtered blood solution to 0° C., add while stirring one-fourth vol. of alcohol also cooled, and allow to stand a few days at −5° to −10° C. The crystals which separate may be repeatedly recrystallized by dissolving in water of about 35° C., cooling, and adding cooled alcohol as above. Lastly, they are washed with cooled water

[1] Gamgee, Zeitschr. f. Biol., **34**; Lewin, Miethe and Stenger, Pflüger's Arch., **118**; Lewin and Miethe, *ibid.*, **121**.

[2] Piettre and Vila, Compt. rend., **140**; Ville and Derrien, *ibid.*, **140**; Aron, Biochem. Zeitschr., **3**.

containing alcohol (one-quarter vol. alcohol) and dried in vacuum at 0° C. or a lower temperature.[1]

For the preparation of oxyhæmoglobin crystals in small quantities from easily crystallizable blood, it is often sufficient to stir a drop of blood with a little water on a microscope slide and allow the mixture to evaporate so that the drop is surrounded by a dried ring. After covering with a cover-glass, the crystals gradually appear radiating from the ring. These crystals are formed more surely if the blood is first mixed with some water in a test-tube and shaken with ether and a drop of the lower deep-colored liquid treated as above on the slide.

Hæmoglobin, also called REDUCED HÆMOGLOBIN OR PURPLE CRUORIN (STOKES [2]), occurs only in very small quantities in arterial blood, in larger quantities in venous blood, and is nearly the only blood-coloring matter after asphyxiation.

Hæmoglobin is much more soluble than the oxyhæmoglobin, and it can therefore be obtained as crystals only with difficulty. These crystals are as a rule isomorphous with the corresponding oxyhæmoglobin crystals, but are darker, having a shade toward blue or purple, and are decidedly more pleochromatic. The hæmoglobin from horse-blood has also been obtained by UHLIK [3] in hexagonal six-sided plates. Its solutions in water are darker and more violet or purplish than solutions of oxyhæmoglobin of the same concentration. They absorb the blue and the violet rays of the spectrum in a less marked degree, but strongly absorb the rays lying between *C* and *D*. In proper dilution the solution shows a spectrum with one broad, not sharply defined band between *D* and *E*, whose darkest part corresponds to the wave-length $\lambda = 559$. (Spectrum plate 2.) This band does not lie in the middle between *D* and *E*, but is toward the red end of the spectrum, a little over the line *D*. A hæmoglobin solution actively absorbs oxygen from the air and is converted into an oxyhæmoglobin solution.

A solution of oxyhæmoglobin may be easily converted into a solution having the spectrum of hæmoglobin by means of a vacuum, by passing an indifferent gas through it, or by the addition of a reducing substance, as, for example, an ammoniacal ferrous-tartrate solution (STOKES' reduction liquid). If an oxyhæmoglobin solution or arterial blood is kept in a sealed tube, we observe a gradual consumption of oxygen and a reduction of the oxyhæmoglobin into hæmoglobin. If the solution has a proper concentration, a crystallization of hæmoglobin may occur in the tube at lower temperatures (HÜFNER [4]).

[1] In regard to the preparation of oxyhæmoglobin, see also Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.; also the works cited in footnote 1, p. 270; also Schuurmanns-Stekhoven, Zeitschr. f. physiol. Chem., **33**, 296; see also Bohr, Skand. Arch. f. Physiol., **3**.

[2] Philosophical Magazine, **28**, No. 190, Nov., 1864.

[3] Pflüger's Arch., **104**.

[4] Zeitschr. f. physiol. Chem., **4**; see also Uhlik, l. c.

Pseudohæmoglobin. LUDWIG and SIEGFRIED [1] have observed that blood which has been reduced by hyposulphites so completely that the oxyhæmoglobin spectrum disappears and only the hæmoglobin spectrum is seen, yields large amounts of oxygen when exposed to a vacuum. Blood which has been reduced by the passage of a stream of hydrogen through it until the oxyhæmoglobin spectrum disappears acts in the same manner. Hence a loose combination of hæmoglobin and oxygen exists which gives the hæmoglobin spectrum, and this combination is called pseudohæmoglobin by LUDWIG and SIEGFRIED. Pseudohæmoglobin, whose presence has been detected in asphyxiation blood from dogs, is considered by HAMMARSTEN as an intermediate step between hæmoglobin and oxyhæmoglobin on the reduction of the latter. The occurrence of pseudohæmoglobin does not seem to have been positively proven.[2]

Methæmoglobin. This name has been given to a coloring-matter which is easily obtained from oxyhæmoglobin as a transformation product and which has been correspondingly found in transudates and cystic fluids containing blood, in urine in hæmaturia or hæmoglobinuria, and also in urine and blood on poisoning with potassium chlorate, amyl nitrite or alkali nitrite, and many other bodies.

Methæmoglobin does not contain any oxygen in molecular or dissociable combination, but still the oxygen seems to be of importance in the formation of methæmoglobin, because it is formed from oxyhæmoglobin and not from hæmoglobin in the absence of oxygen or oxidizing agents. If arterial blood be sealed up in a tube, it gradually consumes its oxygen and becomes venous, and by this absorption of oxygen a little methæmoglobin is formed. The same occurs on the addition of a small quantity of acid to the blood. By the spontaneous decomposition of blood some methæmoglobin is formed, and by the action of ozone, potassium permanganate, potassium ferricyanide, chlorates, nitrites, nitrobenzene, pyrogallol, pyrocatechin, acetanilide, and certain other bodies on the blood an abundant formation of methæmoglobin takes place.

According to the investigations of HÜFNER, KÜLZ, and OTTO [3] methæmoglobin contains just as much oxygen as oxyhæmoglobin, but it is more strongly combined. By the action of potassium ferricyanide or potassium permanganate upon oxyhæmoglobin first 1 molecule oxygen (i.e., the entire quantity of loosely combined oxygen) is split off, and in the subsequent methæmoglobin formation either two oxygen atoms (HALDANE) or two hydroxyl groups are combined (HÜFNER, v. ZEYNEK [4]). Methæmoglobin solutions are reduced to hæmoglobin by reducing agents. JÄDERHOLM and SAARBACH claim that methæmoglobin is first converted

[1] Arch. f. (Anat. u.) Physiol., 1890; see also Ivo Novi, Pflüger's Archiv, 56.

[2] See Hüfner, Arch. f. (Anat. u.) Physiol., 1894, 140.

[3] See Otto Zeitschr. f. physiol. Chem., 7.

[4] Haldane, Journ. of Physiol., 22; v. Zeynek, Arch. f. (Anat. u.) Physiol., 1899; Hüfner, *ibid.*

into oxyhæmoglobin and then into hæmoglobin by reducing substances, while others (HOPPE-SEYLER and ARAKI [1]) dispute this.

According to HÜFNER and REINBOLD [2] 1 gram methæmoglobin can take up 2.685 cc. nitric oxide.

Methæmoglobin crystallizes, as first shown by HÜFNER and OTTO, in brownish-red needles, prisms, or six-sided plates. It dissolves easily in water; the solution has a brown color and becomes a beautiful red on the addition of alkali. The solution of the pure substance is not precipitated by basic lead acetate alone, but by basic lead acetate and ammonia. The absorption-spectrum of a watery or acidified solution of methæmoglobin is, according to JÄDERHOLM and BERTIN-SANS, very similar to that of hæmatin in acid solution, but is easily distinguished from the latter since, on the addition of a little alkali and a reducing substance, the former passes over to the spectrum of reduced hæmoglobin, while a hæmatin solution under the same conditions gives the spectrum of an alkaline hæmochromogen solution (see below). According to ARAKI and DITTRICH, a neutral or faintly acid methæmoglobin solution shows only one characteristic band, α, between C and D, whose middle corresponds to about $\lambda = 634$. The two bands between D and E are only due to contamination with oxyhæmoglobin (MENZIES, LEWIN, MIETHE and STENGER.[3] Methæmoglobin in alkaline solution shows two absorption-bands which are like the two oxyhæmoglobin bands, but they differ from these in that the band β is stronger than α. By the side of the band α and united with it by a shadow lies a third fainter band between C and D, near to D. (Spectrum Plate 4).

The claims as to the action of sodium fluoride upon hæmoglobin and methæmoglobin are somewhat contradictory.[4]

Crystallized methæmoglobin may be easily obtained by treating a concentrated solution of oxyhæmoglobin with a sufficient quantity of concentrated potassium-ferricyanide solution to give the mixture a porter-brown color. After cooling to 0° C. add one-fourth vol. cooled alcohol and allow the mixture to stand a few days in the cold. The crystals may be easily purified by recrystallizing from water by the addition of alcohol.

Cyanmethæmoglobin (cyanhæmoglobin) is, according to HALDANE, identical with photomethæmoglobin (BOCK), which is produced by the influence of sunlight upon a methæmoglobin solution containing potassium ferricyanide. It was first carefully described by R. KOBERT and obtained in a crystalline form

[1] Jäderholm, Zeitschr. f. Biologie, **16**; Saarbach, Pflüger's Arch., **28**; Araki, Zeitschr. f. physiol. Chem., **14**.

[2] Arch. f. (Anat. u.) Physiol., 1904. Suppl.

[3] Jäderholm, l. c.; Bertin-Sans, Comp. rend., **106**; Dittrich, Arch. f. exp. Path. u. Pharm., **29**; Menzies, Journ. of. Physiol, **17**; Lewin and collaborators, footnote 1, page 274. Important references on methæmoglobin are given by Otto, Pflüger's Arch., **31**.

[4] Piettre and Vila, Compt. rend., **140**; Ville and Derrien, *ibid.*, **140**.

by v. ZEYNEK.[1] It is immediately formed in the cold by the action of a hydrocyanic-acid solution upon methæmoglobin, but is formed by its action upon oxyhæmoglobin only at the body temperature. The neutral or faintly alkaline solutions show a spectrum which is very similar to the hæmoglobin spectrum.

Acid hæmoglobin is a coloring-matter produced by the action of very weak acids upon oxyhæmoglobin, which according to HARNACK [2] is not, as used to be admitted, identical with methæmoglobin.

Carbon-monoxide Hæmoglobin [3] is the molecular combination between 1 molecule of hæmoglobin and 1 molecule of CO, according to HÜFNER,[4] which contains 1.34 cc. of carbon monoxide (at 0° and 760 mm. Hg) for 1 gram hæmoglobin. This combination is stronger than the oxygen combination of hæmoglobin. The oxygen is for this reason easily driven out of oxyhæmoglobin by carbon monoxide, and this explains the poisonous action of this gas, which kills by the expulsion of the oxygen of the blood. In regard to the division of the blood-pigments between the carbon monoxide and oxygen under different partial pressures of both gases in the air, we must refer to the investigations of HÜFNER,[5] whose results are tabulated.

The carbon monoxide can be driven out by a vacuum as well as by passing an indifferent gas or oxygen or nitric oxide through the solution for a long time, and in these cases hæmoglobin, oxyhæmoglobin, or nitric-oxide hæmoglobin are formed. The carbon monoxide is also expelled by potassium ferricyanide and methæmoglobin is formed (HALDANE [6]).

Carbon-monoxide hæmoglobin is formed by saturating blood or a hæmoglobin solution with carbon monoxide, and may be obtained as crystals by the same means as oxyhæmoglobin. These crystals are isomorphous with the oxyhæmoglobin crystals, but are less soluble and more stable, and their bluish-red color is more marked. For the detection of carbon-monoxide hæmoglobin, its absorption-spectrum is of the greatest importance. This spectrum shows two bands which are very similar to those of oxyhæmoglobin, but they occur more toward the violet

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[1] Haldane, Journ. of Physiol., **25**; Bock, Skand. Arch. f. Physiol., **6**; Kobert, Pflüger's Arch., **82**; v. Zeynek, Zeitschr. f. physiol. Chem., **33**. See also Leers, Biochem. Zeitschr., **12**.

[2] Zeitschr. f. physiol. Chem., **26**.

[3] In reference to carbon-monoxide hæmoglobin, see especially Hoppe-Seyler, Med.-chem. Untersuch., 201; Centralbl. f. d. med. Wissensch., 1864 and 1865; Zeitschr. f. physiol. Chem., **1** and **13**.

[4] Arch. f. (Anat. u.) Physiol., 1894. On the dissociation constant of carbon-monoxide hæmoglobin, see *ibid.*, 1895. In regard to the contradictory statements of Saint-Martin and others and their disapproval, see Hüfner, Arch. f. (Anat. u.) Physiol., 1903.

[5] Arch. f. exp. Path. u. Pharm., **48**.

[6] Journ. of Physiol., **22**.

part of the spectrum. The middle of the first band corresponds to $\lambda=570$, and the second to $\lambda=542$ (LEWIN, MIETHE and STENGER). These bands do not change noticeably on the addition of reducing substances; this constitutes an important difference between carbon-monoxide hæmoglobin and oxyhæmoglobin. If the blood contains oxyhæmoglobin and carbon-monoxide hæmoglobin at the same time, we obtain on the addition of a reducing substance (ammoniacal ferro-tartrate solution) a mixed spectrum originating from the hæmoglobin and carbon-monoxide hæmoglobin. Carbon-monoxide hæmoglobin also gives a band in the violet $\lambda=416$.

A great many reactions have been suggested for the detection of carbon-monoxide hæmoglobin in medico-legal cases. A simple and at the same time a good one is HOPPE-SEYLER'S alkali test. The blood is treated with double its volume of caustic-soda solution of 1.3 sp. gr., by which ordinary blood is converted into a dingy brownish mass, which when spread out on porcelain is brown with a shade of green. Carbon-monoxide blood gives under the same conditions a red mass, which if spread out on porcelain shows a beautiful red color. Several modifications of this test have been proposed. Another very good reagent is tannic acid, which gives with dilute normal blood a brownish-green precipitate and with carbon-monoxide blood a pale crimson-red precipitate.[1]

As according to BOHR there are several oxyhæmoglobins, so also, according to BOHR and BOCK,[2] there are several carbon-monoxide hæmoglobins, with different amounts of carbon monoxide. As hæmoglobin can unite with oxygen and carbon dioxide simultaneously, as shown by BOHR and TROUP, so also can it unite with carbon monoxide and carbon dioxide simultaneously and independently of each other.

Carbon-monoxide methæmoglobin has been prepared by WEIL and v. ANREP by the action of potassium permanganate on carbon-monoxide hæmoglobin, but this is contradicted by BERTIN-SANS and MOITESSIER.[3] **Sulphur methæmoglobin** is the name given by HOPPE-SEYLER to that coloring-matter which is formed by the action of sulphuretted hydrogen upon oxyhæmoglobin and which is generally designated *sulphæmoglobin*. The solution has a greenish-red, dirty color, and shows two absorption-bands between C and D. This coloring-matter is claimed to be the greenish color seen on the surface of putrefying flesh. According to HARNACK the conditions are different when H_2S is passed through an oxygen-free solution of hæmoglobin (or carbon-monoxide hæmoglobin). The sulphæmoglobin thus formed shows one band in the red between C and D. According to CLARKE and HURTLEY[4] the formation of sulphæmoglobin takes place after the reduction to hæmoglobin.

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[1] In regard to this test (as suggested by Kunkel) and others we refer to Kostin, Pflüger's Arch., **84**, which contains a very excellent summary of the literature on the subject. See also de Domenicis, Chem. Centralbl., 1908, **2**, p. 66.

[2] Centralbl. f. Physiol., 8, and Maly's Jahresber., **25**.

[3] v. Anrep, Arch. f. (Anat. u.) Physiol., 1880; Sans and Moitessier, Compt. rend., **113**.

[4] Hoppe-Seyler, Med.-chem. Untersuch., 151. See Araki, Zeitschr. f. physiol. Chem., **14**; Harnack, l. c.; Clarke and Hurtley, Journ. of Physiol., **36**.

Carbon-dioxide Hæmoglobin, *Carbohæmoglobin.* Hæmoglobin, according to Bohr and Torup,[1] also forms a molecular combination with carbon dioxide whose spectrum is similar to that of hæmoglobin. According to Bohr there are three different carbohæmoglobins, namely, α-, β-, and γ-carbohæmoglobin, in which 1 gram combines with respectively 1.5, 3, and 6 cc. CO_2 (measured at 0° C. and 760 mm.) at 18° C. and a pressure of 60 mm. mercury. If a hæmoglobin solution is shaken with a mixture of oxygen and carbon dioxide, the hæmoglobin combines loosely with the oxygen as well as with the carbon dioxide, independently of each other, just as if each gas existed alone (Bohr). He considers that the two gases are combined with different parts of the hæmoglobin, that is, the oxygen with the pigment nucleus and the carbon dioxide with the protein component. Bohr has given an equilibrium formula for the carbon-dioxide absorption of hæmoglobin at different carbon-dioxide tensions, and the results obtained on calculation, using this formula, correspond very well with the results obtained directly. Attention must be called to the fact that, as observed by Torup, hæmoglobin is in part readily decomposed by the carbon dioxide with the splitting off of some protein.

Nitric-oxide Hæmoglobin is also a crystalline molecular combination which is even stronger than the carbon-monoxide hæmoglobin. Its solution shows two absorption-bands, which are paler and less sharp than the carbon-monoxide hæmoglobin bands, and they do not disappear on the addition of reducing bodies. Hæmoglobin also forms a molecular combination with acetylene.

Hæmorrhodin is the name given by Lehmann to a beautiful red pigment soluble in alcohol and ether, which is extracted from meat and meat products by boiling alcohol and which seems to be produced by the action of small amounts of nitrites. Another pigment isolated by Lewin [2] from the blood of animals poisoned by phenylhydrazine, has been called *hæmoverdin.* By heating a solution of blood-pigment treated with caustic potash and mixed with alcohol to 60° C. we obtain, according to v. Klaveren, a pigment which he calls *kathæmoglobin,* but called by Arnold,[3] who first obtained it, *neutral hæmatin,* which is produced by the splitting off of a ferruginous complex. This pigment still contains protein, but is poorer in iron than the hæmoglobin or methæmoglobin and probably forms an intermediary product in the conversion of the above into hæmatin.

Decomposition products of the blood-pigments. By its decomposition hæmoglobin yields, as previously stated, a *protein,* which has been

[1] Bohr, Extrait du Bull. de l'Acad. Danoise, 1890; Centralbl. f. Physiol., **4** and **17**; Torup, Maly's Jahresber., **17**.

[2] K. B. Lehmann, Sitzungsber. d. phys.-med. Gesellsch. Würzburg, 1899; Lewin, Compt. rend., **133**.

[3] v. Klaveren, Zeitschr. f. physiol. Chem., **33**; Arnold, *ibid.*, **29**.

called *globin* (PREYER, SCHULZ), and a ferruginous *pigment* as chief products. According to LAWROW 94.09 per cent protein, 4.47 per cent hæmatin, and 1.44 per cent other bodies are produced in this decomposition. The globin, which was isolated and studied by SCHULZ,[1] differs from most other proteins by containing a high amount of carbon, 54.97 per cent., with 16.98 per cent of nitrogen. It is insoluble in water, but very easily soluble in acids or alkalies. It is not dissolved by ammonia in the presence of ammonium chloride. Nitric acid precipitates it in the cold, but not when warm. It may be coagulated by heat, but the coagulum is readily soluble in acids. Because of these reactions it is considered as a histone by SCHULZ.

On hydrolytic cleavage globin (from horse-blood) yields, according to ABDERHALDEN,[2] the ordinary cleavage products of the proteins and especially leucine, 29 per cent. It is also important to call attention to the large amount of histidine, 10.96 per cent, while the quantities of arginine and lysine were only 5.42 and 4.28 per cent respectively.

The pigment split off is different, depending upon the conditions under which the cleavage takes place. If the decomposition takes place in the absence of oxygen, a coloring-matter is obtained which is called by HOPPE-SEYLER *hæmochromogen*, by other investigators (STOKES) *reduced hæmatin*. In the presence of oxygen, hæmochromogen is quickly oxidized to hæmatin, and there is therefore obtained in this case *hæmatin* as a colored decomposition product. As hæmochromogen is easily converted by oxygen into hæmatin, so this latter may be reconverted into hæmochromogen by reducing substances.

Hæmochromogen was discovered by HOPPE–SEYLER.[3] It is, according to HOPPE-SEYLER, the colored atomic group of hæmoglobin and of its combinations with gases, and this atomic group is combined with proteins in the pigment. The characteristic absorption of light depends on the hæmochromogen, and it is also this atomic group which binds in the oxyhæmoglobin 1 molecule of oxygen and in the carbon-monoxide hæmoglobin 1 molecule of carbon monoxide with 1 atom of iron. Hæmochromogen is produced in an alkaline solution of hæmatin by the action of reducing bodies. By the reduction of hæmatin in alcoholic ammoniacal solution by means of hydrazine v. ZEYNEK[4] was able to obtain the solid brownish-red ammonia combination.

Hæmochromogen also combines, as HOPPE-SEYLER first showed, with carbon monoxide. This compound, which in aqueous solution gives a spectrum similar to oxyhæmoglobin, has been obtained by PREGL[5] in

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[1] Lawrow, *ibid.*, **26**; Schulz, *ibid.*, **24**; Preyer, Die Blutkristalle, Jena, 1871.
[2] Zeitschr. f. physiol. Chem., **37**; with Baumann, *ibid.*, **51**.
[3] *Ibid.*, **13**.
[4] Zeitschr. f. physiol., Chem., **25**.
[5] *Ibid.*, **44**.

the solid condition as a deep-violet powder which is insoluble in absolute alcohol. In opposition to hæmoglobin the hæmochromogen combines with oxygen more firmly than with carbon monoxide. The assumption of HOPPE-SEYLER that this compound is a combination of 1 molecule hæmochromogen and therefore contains 1 molecule carbon monoxide for 1 molecule of iron has been experimentally substantiated by HÜFNER and KÜSTER and by PREGL.[1]

An alkaline hæmochromogen solution has a beautiful cherry-red color. It shows two absorption-bands, first described by STOKES '(spectrum Plate 6), one of which is dark and whose center corresponds to $\lambda = 556.4$ between *D* and *E*, and a second broader band, less dark, which covers the FRAUNHOFER lines *E* and *b*. The middle of this band corresponds to $\lambda = 526$ to 530 according to LEWIN, MIETHE and STENGER. In acid solution hæmochromogen shows four bands, which, according to JÄDERHOLM,[2] depend on a mixture of hæmochromogen and hæmatoporphyrin (see below), this last formed by a partial decomposition resulting from the action of the acid.

MILROY,[3] from an alcoholic solution of hæmatin containing oxalic acid, after driving out the air by means of hydrogen gas, gradually obtained an acid solution of reduced hæmatin (hæmochromogen) by means of zinc dust. This solution showed one absorption-band between *D* and *E*.

Hæmochromogen may be obtained as crystals by the action of caustic soda on hæmoglobin at 100° C. in the absence of oxygen (HOPPE-SEYLER). By the decomposition of hæmoglobin by acids (of course in the absence of air) we obtain hæmochromogen contaminated with a little hæmatoporphyrin. An alkaline hæmochromogen solution is easily obtained by the action of a reducing substance (STOKES' reduction liquid) on an alkaline hæmatin solution. An ammoniacal solution of hæmatin on reduction with hydrazine yields hæmochromogen very easily. An alcoholic, alkaline hydrazine solution is also recommended by RIEGLER [4] as a reagent for blood-pigments, converting them into hæmochromogen.

Hæmatin, also called OXYHÆMATIN, is sometimes found in old transudates. It is formed by the action of the gastric or pancreatic juices on oxyhæmoglobin, and is, therefore, also found in the feces after hemorrhage in the intestinal canal, and also after a meat diet and food rich in blood. It is stated that hæmatin may occur in urine after poisoning with arseniuretted hydrogen. As shown above, the hæmatin is formed by the decomposition of oxyhæmoglobin, or at least of hæmoglobin, in the presence of oxygen.

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[1] Hüfner, and Küster, Arch. f. (Anat. u.) Physiol., 1904, Suppl. Pregl, l. c.

[2] Nord. Med. Arkiv., **16**.

[3] Journ. of Physiol., **32**.

[4] Zeitschr. f. anal. Chem., **43**.

The views in regard to the composition of hæmatin are rather contradictory, which seems to be due to the fact that the substance hæmin (see below), from which the formula of hæmatin is derived, has a somewhat different composition, dependent upon various conditions. According to HOPPE-SEYLER hæmatin has the formula $C_{34}H_{34}N_4FeO_5$, and from the recent investigations upon hæmin, which will be mentioned below, this formula seems to be now generally accepted. According to this formula 1 atom of iron occurs with every 4 atoms of nitrogen. According to CLOETTA, and also ROSENFELD,[1] hæmatin has the formula $C_{30}H_{34}N_3FeO_3$, with 1 atom of iron for every 3 atoms of nitrogen. The question whether the hæmatins obtained under different conditions are identical or not is still undecided (v. ZEYNEK, EPPINGER[2]).

Hæmatin is very resistant toward boiling concentrated caustic potash as well as toward boiling hydrochloric acid. It dissolves in concentrated sulphuric acid, and is converted into hæmatoporphyrin with the splitting off of iron. On heating dry hæmatin it yields abundant pyrrol. On reduction with tin and hydrochloric acid a body similar to urobilin is formed. As an oxidation product of hæmatin in glacial acetic acid with potassium bichromate or chromium trioxide, KÜSTER obtained the imide of the tribasic hæmatinic acid, $C_8H_9NO_4$, which is also produced on the oxidation of hæmatoporphyrin and bilirubin. On reduction with phosphonium iodide it yields hæmopyrrol (see hæmatoporphyrin).

The imide of the tribasic hæmatinic acid, which is a derivative of maleïc acid and probably has the formula $C_5H_7(COOH)\begin{<}CO\\CO\end{>}NH$, is readily transformed into the anhydride of the tribasic hæmatinic acid, $C_8H_8O_5$, having the probable formula

$$\begin{matrix}CH_3.C.CO\\ \|\\COOH.CH_2.CH_2.C.CO\end{matrix}\!\!>O.$$

On heating the imide with alcoholic ammonia to 130° C. it splits off carbon dioxide, and the imide of the bibasic hæmatinic acid $C_7H_9NO_2$ is obtained. From this imide on saponification with baryta-water we obtain the barium salt of an acid whose anhydride is methyl-ethyl maleïc-acid anhydride,

$$\begin{matrix}C_2H_5.C.CO\\ \|\\CH_3.C.CO\end{matrix}\!\!>O.$$

On heating hæmatinic acid ester with alcoholic ammonia in a tube to 130° KÜSTER obtained a colored product whose bluish-violet aqueous solution gave a spectrum with two bands which in position were similar to the oxyhæmoglobin spectrum. He has also prepared and studied various salts, esters and aniline derivatives of the hæmatinic acids and condensation products of their esters.

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[1] Hoppe-Seyler, Med.-chem. Untersuch., p. 525; Cloetta, Arch. f. exp. Path. u. Pharm., **36**; Rosenfeld, *ibid.*, **40**.

[2] v. Zeynek, Zeitschr. f. physiol. Chem., **49**; P. Eppinger, Unters. über den Blutfarbstoffe Dissert. München, 1907.

Based upon his investigations on the hæmatinic acids and hæmopyrrol, KÜSTER[1] believes that the hæmatin in part contains a group which is readily changed into hæmatinic acid but not into hæmopyrrol, and a part which can be changed into both hæmatinic acid and pyrrol.

PILOTY[2] has published important investigations on the constitution of hæmatin and on the origin of KÜSTER's hæmatinic acid. On warming hæmatoporphyrin with hydrochloric acid and tin chloride he obtained three products, namely, hæmopyrrol, hæmopyrrol carboxylic acid, and desoxyhæmatoporphyrin. Hæmopyrrol seems to be a unit body, and is considered as β-methyl-β-n-propyl-pyrrol. The hæmatinic acid is not formed from hæmopyrrol, but rather from the crystalline hæmopyrrol carboxylic acid, $C_9H_{13}NO_2$, which is formed in addition to hæmopyrrol in the reduction of hæmatoporphyrin, and which is transformed into hæmatinic acid, $C_8H_9NO_4$, on oxidation. The desoxyhæmatoporphyrin, $C_{34}H_{38}N_4O_5$, which differs from the hæmatoporphyrin by containing one atom of oxygen less, yields on further reduction hæmopyrrol, hæmopyrrol carboxylic acid, and hæmatopyrrolidinic acid. This last, which has not been obtained pure, yields on further cleavage and oxidation, hæmatinic acid and a basic oil having a piperidine-like odor. These investigations are in accord with KÜSTER's observations that a part of the hæmatinic acid is relatively easily split, while another part, on the contrary, is only split with difficulty and gradually. They do not, on the contrary, agree with the above statement of KÜSTER that hæmatin contains a group which is readily changed into hæmatinic acid but not into hæmopyrrol, and another group which yields hæmatinic acid as well as pyrrol.

Hæmatin is amorphous, dark brown or bluish black. It may be heated to 180° C. without decomposition; on burning it leaves a residue consisting of iron oxide. It is insoluble in water, dilute acids, alcohol, ether, and chloroform, but it dissolves slightly in warm glacial acetic acid. Hæmatin dissolves in acidified alcohol or ether. It easily dissolves in alkalies, even when very dilute. The alkaline solutions are dichroic; in thick layers they appear red by transmitted light and in thin layers greenish. The alkaline solutions are precipitated by lime- and baryta-water, as also by solutions of neutral salts of the alkaline earths. The acid solutions are always brown.

An acid hæmatin solution (spectrum Plate 4), absorbs the red part of the spectrum only slightly and the violet parts strongly. The solution shows a rather sharply defined band between *C* and *D*, whose position may change with the variety of acid used as a solvent. Between

[1] Beiträge zur Kenntnis des Hämatins, Tübingen, 1896; Ber. d. d. chem. Gesellsch., **27**, **30**, **32**, and **35**; Annal. d. Chem. u. Pharm., **315**, and Zeitschr. f. physiol. Chem., **28**, **40**, **44**, **54**, and **55**.

[2] Annal. d. Chem. u. Pharm., **366**.

D and *F* a second, much broader, less sharply defined band occurs, which by proper dilution of the liquid is converted into two bands. The one between *b* and *F*, lying near *F*, is darker and broader; the other, between *D* and *E*, lying near *E*, is lighter and narrower. Also by proper dilution a fourth very faint band is observed between *D* and *E*, lying near *D*. Hæmatin may thus in acid solution show four absorption-bands; ordinarily one sees distinctly only the bands between *C* and *D* and the broad, dark band—or the two bands—between *D* and *F*. In alkaline solution hæmatin (spectrum Plate 5), shows a broad absorption-band, which lies in greatest part between *C* and *D*, but reaches a little over the line *D* toward the right in the space between *D* and *E*. As the position of the hæmatin bands in the spectrum is quite variable, the exact wave-lengths corresponding thereto cannot be given exactly.

Hæmin, Hæmin Crystals, or Teichmann's Crystals. Hæmin is the hydrochloric-acid ester of hæmatin, and is the starting-point in the preparation of the latter.

Opinions as to the composition of hæmin are just as variable as those for hæmatin, which is partly due to the fact, as shown by Nencki and Zaleski, that the hæmatin, which contains two hydroxyls in the molecule, may form ethers with acids and alkyl radicals, which also yield addition products with indifferent compounds. Thus the hæmin prepared according to Nencki and Sieber's method contains amyl alcohol. Schalfejeff's hæmin, having the formula $C_{34}H_{33}N_4FeO_4Cl$, is supposed to contain an acetyl group, and hence is called acethæmin. Mörner's hæmin, $C_{35}H_{35}N_4FeO_4Cl$, is considered as a monoethyl ether of acethæmin. The investigations of Zaleski, Hetper and Marchlewski, K. Mörner, and especially those of Küster, have given explanations of these conditions. The so-called acethæmin does not contain any acetic-acid radical, hence its name is incorrect. Küster, by a new method of purification and recrystallization, has shown that the older various kinds of hæmins were not chemical individuals, and that we have only one hæmin. This view is now accepted by Mörner and most of the other investigators, and the formula $C_{34}H_{33}O_4N_4FeCl$ is now given to hæmin. Piettre and Vila [1] dispute this formula, and they claim to have prepared chlorine-free hæmin from pure crystalline oxyhæmoglobin.

Hæmin crystals form in large masses a bluish-black powder, but are so small that they can be seen only by aid of the miscrocope. They

[1] Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**; Nencki and Sieber, Arch. f. exp. Path. u. Pharm., **18** and **20**, and Ber. d. d. chem. Gesellsch., **18**; Schalfejeff with Nencki and Zaleski, l. c.; Bialobrzeski, Arch. des scienc. biol. de St. Pétersbourg, 5; K. Mörner, Nord. Med. Arkiv, Festband, 1897, Nos. 1 and 26, and Zeitschr. f. physiol. Chem., **41**; Zaleski, *ibid.*, **37**; Hetper and Marchlewski, *ibid.*, **41** and **42**; Küster, *ibid.*, **40**; Piettre and Vila, Compt. rend., **141**, p. 734.

consist of dark-brown or nearly brownish-black long, rhombic, or spool-like crystals, isolated or grouped as crosses, rosettes, or stellar forms. Cubical crystals may also occur, according to CLOËTTA. They are insoluble in water, dilute acids at the normal temperature, alcohol, ether, and chloroform. They are slightly soluble in glacial acetic acid with heat. They dissolve in acidified alcohol, as also in dilute caustic alkalies or carbonates; and in the last case they form, besides alkali chlorides, soluble hæmatin alkali, from which the hæmatin may be precipitated by an acid. As shown by EPPINGER and then also by v. SIEWERT,[1] crystalline hæmin can be reobtained from the hæmatin.

On shaking with cold aniline and treating first with acetic acid and then with ether, KÜSTER obtained a product, dehydrochloride hæmin, which was poor in the elements of hydrochloric acid, and which again took up HCl and was converted into hæmin. By the action of boiling aniline, hydrogen is driven out and a combination with aniline, without loss of iron, takes place.

The principle of the preparation of hæmin crystals in large quantities is as follows: The washed sediment from the blood-corpuscles is coagulated with alcohol or by boiling after dilution with water and the careful addition of acid. The strongly pressed but not dry mass is rubbed with 90–95 per cent alcohol which has been previously treated with oxalic acid or ½–1-per cent concentrated sulphuric acid, and this is allowed to stand several hours at the temperature of the room. The filtrate is warmed to about 70° C., treated with hydrochloric acid (for each liter of filtrate add 10 cc. 25-per cent hydrochloric acid diluted with alcohol —MÖRNER), and allowed to stand in the cold. The crystals, which separate in one or two days, are first washed with alcohol and then with water. For particulars as to the various methods of preparation and purification we refer the reader to the above-cited works of NENCKI and SIEBER, CLOËTTA, MÖRNER, ROSENFELD, NENCKI and ZALESKI (SHALFEJEFF), and especially to KÜSTER.[2]

Hæmatin is obtained on dissolving the hæmin crystals in very dilute caustic alkali and precipitating with an acid.

In preparing hæmin crystals in small quantities proceed in the following manner: The blood is dried after the addition of a small quantity of common salt, or the dried blood may be rubbed with a trace of the same. The dry powder is placed on a microscope slide, moistened with glacial acetic acid, and then covered with the cover-glass. Add, by means of a glass rod, more glacial acetic acid by applying the drop at the edge of the cover-glass until the space between the slide and the cover-glass is full. Now warm over a very small flame, with the precaution that the acetic acid does not boil and pass with the powder from under the cover-glass. If no crystals appear after the first warming and cooling, warm again, and if necessary add some more acetic acid. After cooling, if the experiment has been properly performed, a number of dark-brown or nearly black hæmin crystals of varying forms will be seen.

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[1] Eppinger, l. c.; v. Siewert, Arch. f. exp. Path. u. Pharm., 58.

[2] Küster, Zeitschr. f. physiol. Chem., 40.

In regard to the preparation and properties of the iodine-, bromine-, and acetone-hæmin we refer to the work of STRZYZOWSKI, MERUNOWICZ and ZALESKI.[1]

By the action of acids upon hæmochromogen, hæmatin, or hæmin, a new iron-free pigment, which was first closely studied by HOPPE-SEYLER and called *hæmatoporphyrin*, is produced. According to the method of preparation hæmatoporphyrins having different solubilities, and whose relation to each other is not perfectly clear, are produced, but all show the same characteristic absorption-spectrum. The best-studied hæmatoporphyrin is the one obtained according to NENCKI and SIEBER'S method, by the action of glacial acetic acid saturated with hydrobromic acid upon hæmin crystals, best at the temperature of the body (NENCKI and ZALESKI [2]).

Hæmatoporphyrin, $C_{16}H_{18}N_2O_3$, or $C_{34}H_{38}N_4O_6$ according to ZALESKI,[3] is a pigment which according to MACMUNN,[4] occurs as a physiological pigment in certain animals. It occurs, as shown by GARROD and SAILLET, as a normal constituent, although only as traces, in human urine. It occurs in greater quantities in human urine after the use of sulfonal (see Chapter XV).

The formation of hæmatoporphyrin from hæmatin can be expressed by the following equation if we start with the above formula for hæmin and ZALESKI'S formula for hæmatoporphyrin:

$$C_{34}H_{33}N_4O_4FeCl + 2HBr + 2H_2O = C_{34}H_{38}N_4O_6 + FeBr_2 + HCl.$$

On heating hæmatoporphyrin it generates an odor of pyrrol. On oxidation with bichromate and glacial acetic acid it yields hæmatinic acid (see page 283). A pigment closely allied to the urinary pigment, urobilin, has been obtained by the action of reducing substances on hæmatoporphyrin (HOPPE-SEYLER, NENCKI and SIEBER, LE NOBEL, MACMUNN). On the administration of hæmatoporphyrin to rabbits, NENCKI and ROTSCHY [5] observed that a part was reduced to a substance similar to urobilin.

Of especial interest are the investigations of NENCKI, MARCHLEWSKI, and ZALESKI [6] upon the reduction products of hæmatoporphyrin and

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[1] Strzyzowski, Therap. Monatsh., 1901 and 1902; Merunowicz and Zaleski, Bull. de l'Acad. d. Scienc. de Cracovie, 1907.

[2] Hoppe-Seyler, Med.-chem. Untersuch., 528; Nencki and Sieber, Monatshefte f. Chem., **9**, and Arch. f. exp. Path. u. Pharm., **18**, **20**, and **24**; Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**.

[3] Zeitschr. f. physiol. Chem., **37**, 54.

[4] Journ. of Physiol., **7**.

[5] Hoppe-Seyler, l. c., 523; Le Nobel, Pflüger's Arch., **40**; MacMunn, Proc. Roy. Soc., **30**, and Journ. of Physiol., **10**; Nencki and Rotschy, Monatshefte f. Chem., **10**.

[6] See footnote 1, page 269.

their relation to the chlorophyll derivatives. By the action of glacial acetic acid containing HI and of iodophosphonium upon hæmin or hæmochromogen NENCKI and ZALESKI obtained a markedly characteristic pigment, *mesoporphyrin*, having the formula $C_{16}H_{18}N_2O_2$, or, according to ZALESKI,[1] $C_{34}H_{38}N_4O_4$, and which stands in a certain measure between hæmatoporphyrin, $C_{16}H_{18}N_2O_3$, and the chlorophyll derivative *phylloporphyrin*, $C_{16}H_{18}N_2O$, which is very similar to hæmatoporphyrin. By the action of the same reducing agent upon hæmin or hæmochromogen, but under other conditions, we obtain *haemopyrrol*, $C_8H_{13}N$, a colorless oil, which in the air gradually changes into urobilin. Hæmopyrrol is produced by the action of the same reducing agents upon the chlorophyll derivative *phyllocyanin* (NENCKI and MARCHLEWSKI), which, as above remarked, shows a close relation between the blood-pigment and chlorophyll.

We have numerous investigations by NENCKI and ZALESKI, KÜSTER and HAAS, MARCHLEWSKI and BURACZEWSKI and ST. MOSTOWSKI, RETINGER, GOLDMANN and HETPER[2] on hæmopyrrol. These investigations have not led to any conclusive results, but show that hæmopyrrol is not a unit body. KÜSTER obtained methyl-ethyl maleinamide as an oxidation product and he considers hæmopyrrol as a mixture of two methyl-ethyl pyrrols. According to MARCHLEWSKI it is, on the contrary, a mixture of several substances whose chief constituent is methyl-propyl pyrrol.

Hæmatoporphyrin is, according to NENCKI and SIEBER, isomeric with the bile-pigment bilirubin, and like this latter gives a play of colors—green, blue, and yellow—when treated with fuming nitric acid.

The hydrochloric-acid compound crystallizes in long brownish-red needles. If the solution in hydrochloric acid is nearly neutralized with caustic soda and then treated with sodium acetate, the pigment separates out as amorphous, brown flakes not readily soluble in amyl alcohol, ether, and chloroform, but readily soluble in ethyl alcohol, alkalies, and dilute mineral acids. The compound with sodium crystallizes as small tufts of brown crystals. The acid alcoholic solutions have a beautiful purple color, which becomes violet-blue on the addition of large quantities of acid. The alkaline solution has a beautiful red color, especially when not too much alkali is present.

An alcoholic solution of hæmatoporphyrin, acidulated with hydrochloric or sulphuric acid, shows two absorption-bands (spectrum plate, 7), one of which is fainter and narrower and lies between *C* and *D*, near

[1] The works of Küster and collaborators may be found in Ber. d. d. chem. Gesellsch., **37** and **40**, and Zeitschr. f. physiol. Chem., **55**, and of Marchlewski and co-workers in Zeitschr. f. physiol. Chem., **43**, **45**, **51**, **56**, and Biochem. Zeitschr., **10**.

[2] See footnote 1.

D. The other is much darker, sharper, and broader, and lies midway between *D* and *E*. An absorption extends from these bands toward the red, terminating with a dark edge, which may be considered as a third band between the other two.

A dilute alkaline solution shows four bands, namely, a band between *C* and *D*; a second, broader band surrounding *D* and with the greater part between *D* and *E*; a third between *D* and *E*, nearly at *E*; and lastly, a fourth broad and dark band between *b* and *F*. On the addition of an alkaline zinc-chloride solution the spectrum changes more or less rapidly,[1] and finally a spectrum is obtained with only two bands, one of which surrounds *D* and the other lies between *D* and *E*. If an acid hæmatoporphyrin solution is shaken with chloroform, a part of the pigment is taken up by the chloroform, and this solution often shows a five-banded spectrum with two bands between *C* and *D*. The position of the hæmatoporphyrin bands in the spectrum differ with the various methods of preparation and other conditions, so that they do not correspond to the same wave length. These facts coincide well with the recent investigations of A. SCHULZ;[2] according to which the appearance of the spectrum is not only dependent upon the reaction but also upon the character of the solvent and the method of preparation.

In regard to the preparation of hæmatoporphyrin, see HOPPE-SEYLER-THIERFELDER'S Handbuch, 8. Aufl., and the works cited on page 288.

Hæmatinogen is a ferruginous pigment so named by FREUND,[3] which he obtained by carefully extracting blood with alcohol containing hydrochloric acid. It is closely related to hæmatin, but is not sufficiently characteristic and is not considered as a cleavage product.

A question of great interest is whether it is possible to produce the blood-pigment from its cleavage products. In this respect certain recent investigations are interesting. ZALESKI obtained from mesoporphyrin hydrochloride dissolved in 80 per cent acetic acid saturated with NaCl and heated to 50–70°, a hæmin-like pigment by the addition of a solution of iron in acetic acid, and this pigment had a spectrum in acid solution very similar to that of hæmatin, although not identical with it. ZALESKI considers this pigment as a hydrogenized hæmin. A regeneration of hæmatin from hæmatoporphyrin has been performed by LAIDLAW. If hæmatoporphyrin is dissolved in dilute ammonia and warmed with STOKES' solution and hydrazine hydrate, iron is taken up again and hæmochromogen is produced, which is changed into hæmatin by shaking with air. According to HAM and BALEAN,[4] it is possible to produce hæmo-

[1] See Hammarsten, Skand. Arch. f. Physiol., 3, and Garrod, Journ. of Physiol., 13.

[2] Arch. f. (Anat. u.) Physiol., 1904, Suppl.

[3] Wien. klin. Wochenschr., 1903.

[4] Zaleski, Zeitschr. f. physiol. Chem., 43; Laidlaw, Journ. of Physiol., 31; Ham and Balean, *ibid.*, 32.

globin from hæmochromogen and globin, and it is indeed possible that other proteins can replace globin in this formation.

Hæmatoidin, thus called by VIRCHOW, is a pigment which crystallizes in orange-colored rhombic plates, and which occurs in old blood extravasations, and whose origin from the blood-coloring matters seems to be established (LANGHANS, CORDUA, QUINCKE, and others [1]). A solution of hæmatoidin shows no absorption-bands, but only a strong absorption from the violet to the green (EWALD [2]). According to most observers, hæmatoidin is identical with the bile-pigment bilirubin. It is not identical with the crystallizable lutein from the *corpora lutea* of the ovaries of the cow (PICCOLO and LIEBEN,[3] KÜHNE and EWALD).

In the detection of the above-described blood-coloring matters the spectroscope is the only entirely trustworthy means of investigation. If it is only necessary to test for blood in general and not to determine definitely whether the coloring-matter is hæmoglobin, methæmoglobin or hæmatin, then the preparation of hæmin crystals is an absolutely positive test. In regard to the detection of blood in urine see Chapter XV and for the detection of blood in intestinal contents, in pathological fluids and in chemico-legal cases we must refer the reader to more extended text-books.

The methods proposed for the quantitative estimation of the blood-coloring matters are partly chemical and partly physical.

Among the chemical methods to be mentioned is the incineration of the blood and the determination of the amount of iron contained in the ash, from which the amount of hæmoglobin may be calculated. JOLLES [4] has suggested a clinical method based on this procedure.

The physical methods consist either of colorimetric or of spectroscopic investigations.

The principle of HOPPE-SEYLER'S *colorimetric method* is that a measured quantity of blood is diluted with an exactly measured quantity of water until the diluted blood solution has the same color as a pure oxyhæmoglobin solution of a known strength. The amount of coloring-matter present in the undiluted blood may be easily calculated from the degree of dilution. In the colorimetric testing we use a glass vessel with parallel sides containing a layer of liquid 1 cm. thick (HOPPE-SEYLER'S hæmatinometer). The use of HOPPE-SEYLER'S colorimetric double pipette is more advantageous. Other good forms of apparatus have been constructed by GIACOSA and ZANGERMEISTER.[5] Instead of an oxyhæmoglobin

[1] A comprehensive review of the literature pertaining to hæmatoidin may be found in Stadelmann, Der Icterus, etc., Stuttgart, 1891, pp. 3 and 45.

[2] Zeitschr. f. Biologie, **22**, 475.

[3] Cit. from Gorup-Besanez, Lebrbuch d. physiol. Chem., 4. Aufl., 1878.

[4] Jolles, Pflüger's Arch., **65**; Monatshefte f. Chem., **17**. See also Oerum, Zeitschr. f anal. Chem., **43**; and the works cited in Maly's Jahresber., **33**.

[5] F. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **16**; G. Hoppe-Seyler, *ibid.*, **21**; Winternitz, *ibid.*; Giacosa, Maly's Jahresber., **26**; Zangermeister, Zeitschr. f. Biologie, **33**.

solution we now generally use a carbon-monoxide hæmoglobin solution as a standard liquid because it may be kept for a long time. The blood solution in this case is saturated with carbon monoxide.[1]

The quantitative estimation of the blood-coloring matters by means of the spectroscope may be done in different ways, but at the present time the *spectrophotometric* method is chiefly used, and this seems to be the most reliable. This method is based on the fact that the extinction coefficient of a colored liquid for a certain region of the spectrum is directly proportional to the concentration, so that $C:E=C_1:E_1$, when C and C_1 represent the different concentrations and E and E_1 the corresponding coefficients of extinction. From the equation $\frac{C}{E}=\frac{C_1}{E_1}$, it follows that for one and the same pigment this relation, which is called the *absorption ratio*, must be constant. If the absorption ratio is represented by A, the determined extinction coefficient by E, and the concentration (the amount of coloring-matter in grams in 1 cc.) by C, then $C=AE$.

Different forms of apparatus have been constructed (Vierordt and Hüfner[2]) for the determination of the extinction coefficient, which is equal to the negative logarithm of those rays of light which remain after the passage of the light through a layer 1 cm. thick of an absorbing liquid. In regard to this apparatus the reader is referred to other text-books.

For purposes of control the extinction coefficients are determined in two different regions of the spectrum. Hüfner has selected (*a*) the region between the two absorption-bands of oxyhæmoglobin, especially between the wave-lengths 554 $\mu\mu$ and 565 $\mu\mu$, and (*b*) the region of the second band, especially the interval between the wave-lengths 531.5 $\mu\mu$ and 542.5 $\mu\mu$. The constants or the absorption ratio for these two regions of the spectrum are designated by Hüfner by A and A'. Before the determination the blood must be diluted with water, and if the proportion of dilution of the blood be represented by V, then the concentration or the amount of coloring-matter in 100 parts of the undiluted blood is

$$C=100.\ V.\ A.\ E \text{ and}$$
$$C=100.\ V.\ A'.\ E'.$$

The absorption ratio or the constants in the two above-mentioned regions of the spectrum have been determined for oxyhæmoglobin, hæmoglobin, carbon monoxide hæmoglobin, and methæmoglobin, as follows:

Oxyhæmoglobin . . . . . A_o = 0.002070 and A'_o = 0.001312
Hæmoglobin . . . . . A_r = 0.001354 and A'_r = 0.001778
Carbon-monoxide hæmoglobin . . A_c = 0.001383 and A'_c = 0.001263
Methæmoglobin . . . . . A_m = 0.002077 and A'_m = 0.001754

The quantity of each coloring matter may be determined in a mixture of two blood-coloring matters by this method; this is of special importance in the determination of the quantity of oxyhæmoglobin and hæmoglobin present in blood at the same time.

In order to facilitate these determinations, Hüfner[3] has worked out tables which give the relation between the two pigments existing in a solution contain-

[1] See Haldane, Journ. of Physiol., **26**.

[2] See Vierordt, Die Anwendung des Spektralapparates zu Photometrie, etc. (Tübingen, 1873), and Hüfner, Arch. f. (Anat. u.) Physiol., 1894, and Zeitschr. f. physiol. Chem., **3**; v. Noorden, *ibid.*, **4**; Otto, Pflüger's Arch., **31** and **36**.

[3] Arch. f. (Anat. u.) Physiol., 1900.

ing oxyhæmoglobin and another pigment (hæmoglogbin, methæmoglobin, or carbonmonoxide hæmoglobin), and thus allowing of the calculation of the absolute quantity of each pigment.

Among the many apparatus constructed for clinical purposes for the quantitative estimation of hæmoglobin, FLEISCHL'S *hæmometer*, which has undergone numerous modifications, HÉNOCQUE'S *hæmatoscope*, and SAHLI'S *hæmometer* are to be specially mentioned. In regard to these apparatus we must refer to larger hand-books and text-books on clinical methods.

Many other pigments are found besides the often-occurring hæmoglobin in the blood of invertebrates. In a few Arachnidæ, Crustacea, Gasteropodæ and Cephalopodæ a body analogous to hæmoglobin, containing copper, *hæmocyanin*, has been found by FREDERICQ. By the taking up of loosely bound oxygen this body is converted into blue *oxyhæmocyanin*, and by the escape of the oxygen becomes colorless again. According to HENZE 1 gram hæmocyanin combines with about 0.4 cc. oxygen. It is crystalline and has the following composition: C 53.66; H 7.33; N 16.09; S 0.86; Cu. 0.38; O 21.67 per cent. On hydrolytic cleavage with hydrochloric acid HENZE found the following division of the nitrogen in hæmocyanin: Of the total nitrogen 5.78 per cent was split off as ammonia, 2.67 per cent as humus nitrogen, 27.65 per cent as diamino nitrogen, and 63.39 per cent as monamino nitrogen. He found no arginine in the cleavage products, but could detect histidine, lysine, tyrosine, and glutamic acid. A coloring-matter called *chlorocruorin* by LANKESTER is found in certain Chætopodæ. *Hæmerythrin*, so called by KRUKENBERG but first observed by SCHAWLBE, is a red coloring matter from certain Gephyrea. Besides hæmocyanin we find in the blood of certain Crustacea the red coloring matter *tetronerythrin* (HALLIBURTON), which is also widely spread in the animal kingdom. *Echinochrom*, so named by MACMUNN,[1] is a brown coloring matter occurring in the perivisceral fluid of a variety of echinoderms.

The *quantitative constitution of the red blood-corpuscles*. The amount of water varies in different varieties of blood-corpuscles between 570–644 p. m., with a corresponding amount, 430–356 p. m., of solids. The chief mass, about $\frac{8}{10}$–$\frac{9}{10}$, of the dried substance consists of hæmoglobin (in human and mammalian blood).

According to the analyses of HOPPE-SEYLER[2] and his pupils, the red corpuscles contain in 1000 parts of the dried substance:

	Hæmoglobin.	Protein.	Lecithin.	Cholesterin.
Human blood.....	868–944	122–51	7.2–3.5	2.5
Dog's "	865	126	5.9	3.6
Goose's "	627	364	4.6	4.8
Snake's "	467	525	...	...

ABDERHALDEN found the following composition for the blood-corpuscles from the domestic animals investigated by him: Water, 591.9–644.3 p. m.; solids 408.1–335.7 p. m.; hæmoglobin, 303.3–331.9 p. m.;

[1] Fredericq, Extrait des Bulletins de l'Acad. Roy. de Belgique (2), **46**, 1878; Lankester, Journ. of Anat. and Physiol., **2** and **4**; Henze, Zeitschr. f. physiol. Chem., **33** and **43**; Krukenberg, see Vergl. physiol. Studien, Reihe 1, Abt. 3, Heidelberg, 1880; Halliburton, Journal of Physiol., **6**; MacMunn, Quart. Journ. Microsc. Science, 1885.

[2] Med.-chem. Untersuch., 390 and 393.

protein, 5.32 (dog)–78.5 p. m. (sheep); cholesterin, 0.388 (horse)–3.593 p. m. (sheep); and lecithin, 2.296 (dog)–4.855 p. m.

Of special interest is the varying proportion of the hæmoglobin to the protein in the nucleated and in the non-nucleated blood-corpuscles. These last are much richer in hæmoglobin and poorer in protein than the former.

The amount of mineral bodies in various species of animals is different. According to BUNGE and ABDERHALDEN the red corpuscles from the pig, horse, and rabbit contain no soda, while those from man, the ox, sheep, goat, dog, and cat are relatively rich in soda. In the five last-mentioned species the amount of soda was 2.135–2.856 p. m. The quantity of potash was 0.257 (dog)–0.744 p. m. (sheep). In the horse, pig, and rabbit the quantity of potash was 3.326 (horse)–5.229 p. m. (rabbit). Human blood-corpuscles contain, according to WANACH, about five times as much potash as soda, on an average 3.99 p. m. potash and 0.75 p. m. soda. The nucleated erythrocytes of the frog, toad, and turtle also contain, according to BOTTAZZI and CAPPELLI,[1] considerably more potassium than sodium. Lime is claimed to be absent in the blood-corpuscles, and magnesia occurs only in small amounts: 0.016 (sheep)–0.150 p. m. (pig). The blood-corpuscles of all animals investigated contain chlorine, 0.460–1.949 p. m. (both in horse), generally 1 to 2 p. m., and also phosphoric acid. The amount of inorganic phosphoric acid shows great variation: 0.275 (sheep)–1.916 p. m. (horse). All of the above figures are calculated on the fresh, moist blood-corpuscles.

By quantitative determinations of the swelling and shrinking of the cells under the influence of NaCl solutions of various concentration or of serum of various dilutions, HAMBURGER has attempted to determine for the erythrocytes, as well as the leucocytes, the percentage relationship between the two chief constituents of the cells (the frame and the intracellular fluid). He found that the volume of the frame-substance for both varieties of blood-corpuscles of the horse was equal to 53–56.1 per cent. The volume for the red blood-corpuscles was for the rabbit 48.7–51; hen, 52.4–57.7, and for the frog, 72–76.4 per cent. KOEPPE has raised objections to these determinations.[2]

The White Blood-corpuscles and the Blood-plates.

The White Blood-corpuscles, also called LEUCOCYTES or Lymphoid Cells, are of different kinds, and ordinarily we differentiate between the small forms poor in protoplasm, called lymphocytes, and the larger, granular, often more nucleated forms, called leucocytes. The polynuclear leucocytes occur in greater abundance in the blood than the lymphocytes. In human and mammalian blood, most of the white blood-corpuscles are larger than the red blood-corpuscles. They also have a lower specific

[1] Bunge, Zeitschr. f. Biologie, **12**, and Abderhalden, Zeitschr. f. physiol. Chem., **23** and **25**; Wanach, Maly's Jahresber., **18**, 88; Bottazzi and Cappelli, Arch. Ital. de Biologie, **32**.

[2] Hamburger, Arch. f. (Anat. u.) Physiol., 1898; Koeppe, *ibid.*, 1899 and 1900.

gravity than the red corpuscles, move in the circulating blood nearer to the walls of the blood-vessels, and also have a slower motion.

The number of white blood-corpuscles varies not only in the different blood-vessels, but also under different physiological conditions. On an average there is only 1 white corpuscle for 350–500 red corpuscles. According to the investigations of ALEX. SCHMIDT[1] and his pupils, the leucocytes are destroyed in great part on the discharge of the blood before and during coagulation, so that discharged blood is much poorer in leucocytes than the circulating blood. The correctness of this statement has been denied by other investigators.

From a histological standpoint we generally, as above indicated, discriminate between the different kinds of colorless blood-corpuscles. Chemically considered, however, there is no known essential difference between them, and what little we do know chemically is chiefly in connection with the leucocytes. With regard to their importance in the coagulation of fibrin, ALEX. SCHMIDT and his pupils distinguish between the leucocytes which are destroyed in the coagulation and those which are not. The last mentioned give with alkalies or common-salt solutions a slimy mass; the first do not show such behavior.

The protoplasm of the leucocytes has during life amœboid movements which serve partly to make possible the wandering of the cells, and partly to aid in the absorption of smaller grains or foreign bodies. On these grounds the occurrence of *myosin* in them has been admitted even without any special proof thereof. We know nothing with positiveness whether in the leucocytes, or in the cells, in general, globulins occur with traces of albumins, because cell constituents which used to be called globulins have on more careful investigation been found to be nucleoalbumins or nucleoproteins. The substance observed by HALLIBURTON,[2] and occurring in all cells, which coagulates at 47 to 50° C., is considered as a true globulin. ALEX. SCHMIDT claims to have found serglobulin in equine-blood leucocytes which have been washed with ice-cold water.

The proteins of the leucocytes as well as the cells in general are chiefly compound proteins. For the present it is impossible to state to what extent the nucleoalbumins occur in leucocytes or cells, because in the past no careful differentiation was made between the nucleoalbumins and nucleoproteins. The nucleoproteins are without any doubt the chief constituents of the protoplasm of the white blood-corpuscles, and one of these it seems is identical with the so-called hyaline substance of ROVIDA, which yields a slimy mass when treated with alkalies or NaCl solutions and which occur in pus-cells.

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[1] Pflüger's Arch., **11** and Krüger, Arch. f. exp. Path. u. Pharm., **51**.

[2] See Halliburton, On the chem. Physiol. of the animal cell. King's College, London, Physiol. Labor. Collected papers, 1893.

On digesting the leucocytes with water, a solution of a protein body is obtained which can be precipitated by acetic acid and which forms the chief mass of the leucocytes. This substance, which is undoubtedly concerned in the coagulation of the blood, has been described under different names, such as *tissue fibrinogen* (Wooldridge) *cytoglobin* and *präglobulin* (Alex. Schmidt) or *nucleohistone* (Kossel and Lilienfeld [1]) and consists, chiefly at least, of nucleoprotein. The ordinary view that this is nucleohistone does not seem to be correct, according to the recent investigations of Bang,[2] and further proof is necessary.

Besides these constituents of the protoplasm of the leucocytes we must also include *lecithin* and especially *phosphatides, cholesterin, glucothionic acid* (in pus corpuscles, Mandel and Levene [3]), *purine bodies* derived from the nuclein substances and *glycogen*. According to Hoppe-Seyler glycogen is a constant constituent of all cells having amœboid movement, and he found it in the colorless blood-corpuscles but not in the non-mobile pus-cells. Nevertheless glycogen has also been found in pus-cells by Salomon [4] and by others. The glycogen found by Huppert, Czerny, Dastre,[5] and others in blood and lymph probably originated from the leucocytes. *Enzymes* also occur in the leucocytes and the proteolytic enzymes are of special importance. According to Opie and Barker [6] two proteolytic enzymes occur in the leucocytes, one of which is active in alkaline solution and occurs in the polynuclear cells while the other is active in acid solution and occurs in the large mononuclear cells. In regard to the other constituents of the leucocytes we refer to Chapter VII, on pus.

The **blood-plates** (Bizzozero), hæmatoblasts (Hayem), whose nature, preformed occurrence, and physiological importance have been much questioned, are pale, colorless, gummy disks, round or somewhat oval in shape, and generally with a diameter one-half or one-third that of the blood-corpuscles. By the action of different reagents the blood-plates are separated into two substances, one of which is homogeneous and non-refractive, while the other is highly refractive and granular. Blood-plates readily stick together and attach themselves to foreign bodies.

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[1] See Wooldridge, Die Gerinnung des Blutes (published by M. v. Frey, Leipzig, 1891); A. Schmidt, Zur Blutlehre, Leipzig, 1892; Lilienfeld, Zeitschr. f. physiol. Chem., **18**.

[2] I. Bang, Studier over Nukleoproteider, Kristiania, 1902.

[3] Biochem. Zeitschr., **4**.

[4] In regard to the literature on Glycogen see Chapter VIII.

[5] Huppert, Centralbl. f. Physiol., **6**, 394; Czerny, Arch. f. exp. Path. u. Pharm., **31**; Dastre, Compt. rend., **120**, and Arch. de Physiol. (5), **7**. See also Hirschberg, Zeitschr. f. klin. Med., **54**.

[6] See Erben, Jochmann and E. Müller, Jochmann and Lockemann, Hofmeister's Beiträge, **11**, which contains the literature. Opie, Journ. of exper. Medicine, **8**; with Barker, *ibid.*, **9**.

According to the researches of KOSSEL and of LILIENFELD [1] the blood-plates consist of a chemical combination between protein and nuclein, and hence they are also called *nuclein-plates* by LILIENFELD, and are considered as derivatives of the cell nucleus. It seems certain that the blood-plates have some connection with the coagulation of blood. The views on this question, and especially in regard to the manner in which these plates act in coagulation, are unfortunately very divergent.

III. THE BLOOD AS A MIXTURE OF PLASMA AND BLOOD-CORPUSCLES.

The blood in itself is a thick, sticky, light or dark red liquid, opaque even in thin layers, having a salty taste and a faint odor differing in different kinds of animals. On the addition of sulphuric acid to the blood the odor is more pronounced. In adult human beings the specific gravity ranges between 1.045 and 1.075. It has an average of 1.058 for grown men and a little less for women. LLOYD JONES found that the specific gravity is highest at birth and lowest in children when about two years old and in pregnant women. The determinations of LLOYD JONES, HAMMERSCHLAG,[2] and others show that the variation of the specific gravity, dependent upon age and sex, corresponds to the variation in the quantity of hæmoglobin.

The determination of the specific gravity is most accurately done by means of the pyknometer. For clinical purposes, where only small amounts are available, it is best to proceed by the method as suggested by HAMMERSCHLAG. Prepare a mixture of chloroform and benzene of about 1.050 sp. gr. and add a drop of the blood to this mixture. If the drop rises to the surface then add benzene, and if it sinks add chloroform. Continue this until the drop of blood suspends itself midway and then determine the specific gravity of the mixture by means of an areometer. This method is not strictly accurate and must be performed quickly. In regard to the necessary details refer to ZUNTZ and A. LEVY.[3]

The reaction of the blood is alkaline toward litmus. The quantity of alkali, calculated as Na_2CO_3, in fresh, non-defibrinated blood from the dog, horse, and man, is, according to LOEWY, 4.93, 4.43, and 5.95 p. m. respectively. According to STRAUSS the average for normal human blood may be calculated as about 4.3 p. m. Na_2CO_3. Quantities below 3.3 p. m. and above 5.3 p. m. are, according to him, to be considered as

[1] In regard to the literature of the blood-plates, see Lilienfeld, Arch. f. (Anat. u.) Physiol., 1892, and "Leukocyten und Blutgerinnung," Verhandl. d. physiol. Gesellsch. zu Berlin, 1892; and also Mosen, Arch. f. (Anat. u.) Physiol., 1893, and Maly's Jahresber., **30** and **31**.

[2] Lloyd Jones, Journ. of Physiol., 8; Hammerschlag, Wien. klin. Wochenschrift, 1890, and Zeitschr. f. klin. Med., **20**.

[3] Zuntz, Pflüger's Arch., **66**; Levy, Proceed. Roy. Soc., **71**.

pathological. v. JAKSCH found the quantity of alkali in man to vary between 3.38 and 3.90 p. m., and BRANDENBURG found 3 p. m. NaOH (=3.98 p. m. Na_2CO_3). The alkaline reaction diminishes outside of the body, and indeed the more quickly the greater the original alkalinity of the blood. This depends on the formation of acid in the blood, in which the red-blood corpuscles seem to take part in some way or another. After excessive muscular activity the alkalinity is diminished (PEIPER, COHNSTEIN), and it is also decreased after the continuous ingestion of acids (LASSAR, FREUDBERG [1]). According to the investigations of ALLERS and BONDI [2], on poisoning rabbits with hydrochloric acid, the relation of the lime to the other bases changes by a relative increase in the lime.

Numerous investigations have been made in regard to the alkalinity of the blood in disease, but as there is at present no trustworthy method for estimating the alkalinity of the blood, and as the results are dependent upon the indicator used, these investigations, as also the claims in regard to the physiological alkalinity, require further substantiation.[3] Attention must also be called to what was stated (page 264) in regard to the determination of the alkalinity of blood-serum—that determinations are made only of the titratable alkali and not of the true alkalinity caused by hydroxyl ions. This alkalinity, as previously remarked, is so very slight that the blood is considered as a nearly neutral fluid. As explained by L. HENDERSON,[4] the carbon dioxide, the alkali carbonate and the mono- and di-phosphates are of especially great importance for this condition as well as for the regulation of the reaction of the blood.

The alkali of the blood exists in part as alkaline salts, carbonate and phosphate, and partly in combination with protein or hæmoglobin. The first are often spoken of as readily diffusible alkalies, while the others are not or are only diffusible with difficulty (see page 261). The quantity of the first in human blood is about one-fifth of the total alkali

[1] Loewy, Pflüger's Arch., **58**, which also contains the references to the literature; H. Strauss, Zeitschr. f. klin. Med., **30**; v. Jaksch, *ibid.*, **13**; Peiper, Virchow's Arch., **116**; Cohnstein, *ibid.*, **130**, which also cites the works of Minkowski, Zuntz, and Geppert; Freudberg, *ibid.*, **125**. See also Weiss, Zeitschr. f. physiol. Chem., **38**; Brandenburg, Zeitschr. f. klin. Med., **45**.

[2] Biochem. Zeitschr., **6**.

[3] In regard to the methods for the estimation of the alkalinity see, besides the above-mentioned authors, v. Jaksch, Klin. Diagnostik; v. Limbeck, Wien. med. Blätter, **18**; Wright, The Lancet, 1897; Biernacki, Beiträge zur Pneumatologie, etc., Zeitschr. f. klin. Med., **31** and **32**; Hamburger, Eine Methode zur Trennung, etc., Arch. f. (Anat. u.) Physiol, 1898. See also Maly's Jahresber., **29**, **30**, and **31**; Salaskin and Pupkin, Zeitschr. f. physiol. Chem., **42**, and O. Folin, *ibid.*, **43**; Laitinen, Hammarsten's Festschr., 1906; Westenrijk, Arch. f. exp. Path. u. Pharm. Suppl., 1908, Schmiedeberg-Festschrift.

[4] Amer. Journ. of Physiol., **21** (1908).

(Brandenburg). The readily as well as the difficultly diffusible alkali is divided between the blood-corpuscles and plasma, and the blood-corpuscles seem to be richer in difficultly diffusible alkali than the plasma or serum. This division may be changed by the influence of even very small amounts of acid, even of carbonic acid, and also, as shown by Zuntz, Loewy and Zuntz, Hamburger, Limbeck, and Gürber,[1] by the influence of the respiratory exchange of gas. The blood-corpuscles give up a part of the alkali united with protein to the serum by the action of carbon dioxide, hence the serum becomes more alkaline. The equilibrium of the osmotic tension in the blood-corpuscles and in the serum is thus disturbed; the blood-corpuscles swell up because they take up water from the serum, and this then becomes more concentrated and richer in alkali, protein, and sugar. Under the influence of oxygen, the corpuscles take their original form again and the above changes are reversed. The blood-corpuscles for this reason are less biconcave in their small diameter in venous than in arterial blood (Hamburger).

These conditions have been further studied by v. Korányi and Bence,[2] and they have investigated the relation between the changes of the volume of the blood-corpuscles and the electrical conductivity, the refractivity of the serum and the viscosity of the blood. The refraction coefficient of the serum is highest with an increase in the amount of carbon dioxide, while it is lowest when the blood is rich in oxygen and poor in carbon dioxide. They consider this as an action of acid, as a similar rise is observed after the addition of acid, while after the addition of alkali a fall in the refraction cofficient of the serum takes place, and these same changes can be brought about by CO_2 or by a current of oxygen. With an increase in the amount of carbon dioxide, the conductivity of the blood diminishes; the viscosity is, on the other hand, highest when the blood is richest in carbon dioxide. If the CO_2 is driven off by O the viscosity diminishes to a minimum, and on leading in more oxygen it rises again. The changes in viscosity [3] of the blood runs parallel with the volume changes of the blood-corpuscles, and changes in the viscosity, which can be brought about by the removal of carbon dioxide, cause a change in the electric charge of the blood-corpuscles (v. Korányi and Bence).

The color of the blood is red—light scarlet-red in the arteries and dark bluish red in the veins. Blood free from oxygen is dichroic, dark red by reflected light and green by transmitted light. The blood-coloring

[1] Zuntz, in Hermann's Handbuch der Physiol., **4**, Abt. 2; Loewy and Zuntz, Pflüger's Arch., **58**; Hamburger, Arch. f. (Anat. u.) Physiol., 1894 and 1898, and Zeitschr. f. Biologie, **28** and **35**; v. Limbeck, Arch. f. exp. Path. u. Pharm., **35**; Gürber, Sitzungsber. d. phys. med. Gesellsch zu Würzburg, 1895.

[2] Pflüger's Arch., **110**.

[3] In regard to the viscosity of the blood and the literature of the subject, see R. Höber in Oppenheimer's Handb. der Bioch., **2**, p. 12–18

matters occur in the blood-corpuscles. For this reason blood is opaque in thin layers. If the hæmoglobin is removed from the stroma and dissolved by the blood liquid by any of the above-mentioned means (see page 266), the blood becomes transparent and has then a "*lake color.*"[1] Less light is now reflected from its interior, and this laky blood is therefore darker in thicker layers. On the addition of salt solutions to the blood-corpuscles they shrink, more light is reflected, and the color appears lighter. A great abundance of red corpuscles makes the blood darker, while by diluting with serum or by a greater abundance of white corpuscles the blood becomes lighter in appearance. The different colors of arterial and of venous blood depend on the varying quantities of gas contained in these two varieties of blood, or, better, on the different amounts of oxyhæmoglobin and hæmoglobin they contain.

The most striking property of blood consists in its coagulating within a shorter or longer time, but as a rule very shortly after leaving the veins. Different kinds of blood coagulate with varying rapidity; in human blood the first marked sign of coagulation is seen in two to three minutes, and within seven to eight minutes the blood is thoroughly converted into a gelatinous mass. If the blood is allowed to coagulate slowly, the red corpuscles have time to settle more or less before the coagulation, and the blood-clot then shows an upper yellowish-gray or reddish-gray layer consisting of fibrin enclosing chiefly colorless corpuscles. This layer has been called *crusta inflammatoria* or *phlogistica*, because it has been especially observed in inflammatory processes and is considered one of the characteristics of them. This crusta, or "*buffy coat,*" is not characteristic of any special disease, and it occurs chiefly when the blood coagulates slowly or when the blood-corpuscles settle more quickly than usual. A buffy coat is often observed in the slowly coagulating equine blood. The blood from the capillaries is not supposed to have the power of coagulating.

Coagulation is retarded by cooling, by diminishing the oxygen, and by increasing the amount of carbon dioxide, which is the reason that venous blood and to a much higher degree blood after asphyxiation coagulates more slowly than arterial blood. The coagulation may be retarded or prevented by the addition of acids, alkalies, or ammonia, even in small quantities; by concentrated solutions of neutral alkali salts and alkaline earths, alkali oxalates and fluorides; also by egg-albumin, solutions of sugar or gum, glycerin, or much water; also by receiving the blood in oil. Coagulation may be prevented by the injection of a proteose solution or of an infusion of the leech into the circulating blood, but this

[1] R Du Bois-Reymond presents objections to the general use of the above terms in Centralbl f Physiol., 19, p. 65.

infusion also acts in the same way on blood just drawn. Coagulation is also hindered by snake poison (cobra-poison), and bacterial toxines. The coagulation may be facilitated by raising the temperature; by contact with foreign bodies, to which the blood adheres; by stirring or beating it; by admission of air; by diluting with very small amounts of water; by the addition of platinum-black or finely powdered carbon; by the addition of laky blood, which does not act by the presence of dissolved blood-coloring matters, but by the stromata of the blood-corpuscles; and also by the addition of the leucocytes from the lymphatic glands, or of a watery saline extract of the lymphatic glands, testicles, or thymus and various other organs (DELEZENNE, WRIGHT, ARTHUS,[1] and others).

An important question to answer is why the blood remains fluid in the circulation, while it quickly coagulates when it leaves the circulation. The reason why blood coagulates on leaving the body is therefore to be sought for in the influence which the walls of the living and uninjured blood-vessels exert upon it. These views are derived from the observations of many investigators. From the observations of HEWSON, LISTER, and FREDERICQ it is known that when a vein full of blood is ligatured at the two ends and removed from the body, the blood may remain fluid for a long time. BRÜCKE[2] allowed the heart removed from a tortoise to beat at 0° C., and found that the blood remained uncoagulated for some days. The blood from another heart quickly coagulated when collected over mercury. In a dead heart, as also in a dead blood-vessel, the blood soon coagulates, and also when the walls of the vessel are changed by pathological processes.

What then is the influence which the walls of the vessels exert on the liquidity of the circulating blood? FREUND found that the blood remains fluid when collected by means of a greased canula under oil or in a vessel smeared with vaseline. If the blood collected in a greased vessel be beaten with a glass rod previously oiled, it does not coagulate, but it quickly coagulates on beating it with an unoiled glass rod or when it is poured into a vessel not greased. The non-coagulability of blood collected under oil was confirmed later by HAYCRAFT and CARLIER. FREUND found on further investigation that the evaporation of the upper layers of blood or their contamination with small quantities of dust causes a coagulation even in a vessel treated with vaseline. According to FREUND[3] it is this adhesion between the blood and a foreign substance

[1] Delezenne, Arch. de Physiol. (5), 8; Wright, Journ. of Physiol., 28; Arthus, Journ. de Physiol. et Pathol., 4.

[2] Hewson's works, edited by Gulliver, London, 1876, cited from Gamgee, Textbook of Physiol. Chem., 1, 1880; Lister, cited from Gamgee, *ibid.*; Fredericq, Recherches sur la constitution du plasma sanguin, Gand, 1878; Brücke, Virchow's Arch., 12.

[3] Freund, Wien. med. Jahrb., 1886; Haycraft and Carlier, Journ. of Anat. and Physiol., 22.

—and the diseased walls of the vessel also act as such—that gives the impulse toward coagulation, while the lack of adhesion prevents the blood from coagulating. BORDET and GENGOU [1] have also shown that the plasma obtained by centrifuging blood collected in a paraffined vessel, and perfectly free from form-elements, can be kept without coagulating in a paraffined vessel, and that it does coagulate on being transferred to an unparaffined vessel. The adhesion of the plasma to a foreign body may also, in the absence of form-elements, give the impulse to coagulation. That this adhesion of the form-elements is of great importance cannot be denied and is also generally accepted. By this adhesion the form-elements undergo certain changes which seem to stand in a certain relation to the coagulation of the blood.

The views in regard to these changes are, unfortunately, very divergent. According to ALEX. SCHMIDT [2] and the Dorpat school an abundant destruction of the leucocytes, especially polynuclear leucocytes, takes place in coagulation, and important constituents for the coagulation of the fibrin pass into the plasma. A direct relation between the destruction of leucocytes and coagulation is denied by many investigators, while according to other experimenters the essential factor is not a destruction of the leucocytes, but an elimination of constituents from the cells into the plasma. This process is called *plasmoschisis* by LÖWIT.[3] The passage of cell constituents into the plasma before coagulation must not necessarily be considered as a phenomenon of death, as it may just as well be a secretory process (ARTHUS, MORAWITZ, DASTRE [4]). Great importance has also been ascribed to the blood-plates in coagulation, as certain investigators (BIZZOZERO, LILIENFELD, SCHWALBE, MORAWITZ, BÜRKER) found that they cause or accelerate coagulation, while others (PETRONE) on the contrary find a retarding action[5].

WOOLDRIDGE [6] takes a very peculiar position in regard to this question: he considers the form-elements as only of secondary importance in coagulation. As he has found, a peptone-plasma which has been freed from all form-con-

[1] Annal. de l'Institute Pasteur, **17**.

[2] Pflüger's Arch., **11**. The works of Alex. Schmidt are found in Arch. f. Anat. und Physiol., 1861, 1862; Pflüger's Arch., **6**, **9**, **11**, **13**. See especially Alex. Schmidt, Zur Blutlehre (Leipzig, 1892), which also gives the work of his pupils, and Weitere Beiträge zur Blutlehre, 1895.

[3] Wien. Sitzungsber., **89** and **90**, and Prager med. Wochenschr., 1889, referred to in Centralbl. f. d. med. Wissensch., **28**, 265.

[4] Morawitz, Hofmeister's Beiträge, **5**; Arthus, Compt. rend. soc. biolog., **55**; Dastre, *ibid.*, **55**.

[5] See foot-note 1, p. 296. Also Schwalbe, Unters. z. Blutgerinnung, etc., Braunschweig, 1900; Morawitz, Deutsch. Arch. f. klin. Med., **79**, and Hofmeister's Beiträge, **4** and **5**; Bürker, Pflüger's Arch., **102**, and Centralbl. f. Physiol., **21**; Petrone, Maly's Jahresber., **31**, p. 170.

[6] Die Gerinnung des Blutes (published by M. v. Frey, Leipzig, 1891).

stituents by means of centrifugal force yields abundant fibrin when it is not separated from a substance which precipitates on cooling. This substance, which WOOLDRIDGE has called A-fibrinogen, seems to all appearances to be a nucleoproteid, which, according to the unanimous view of several investigators, originates from the form-elements of the blood, either the blood-plates or the leucocytes, and the generally accepted view as to the great importance of the form-elements in the coagulation of the blood is not really contrary to WOOLDRIDGE'S experiments.

There is great diversity of opinion in regard to those bodies which are eliminated from the form-elements of the blood before and during coagulation.

According to ALEX. SCHMIDT the leucocytes, like all cells, contain two chief groups of constituents, one of which accelerates coagulation, while the other retards or hinders it. The first may be extracted from the cells by alcohol, while the other cannot be extracted. Blood-plasma contains only traces of thrombin, according to SCHMIDT, but does contain its antecedent, prothrombin. The bodies which accelerate coagulation are neither thrombin nor prothrombin, but they act in this wise in that they split off thrombin from the prothrombin. On this account they are called *zymoplastic substances* by ALEX. SCHMIDT. The nature of these bodies is unknown, and SCHMIDT has given no opinion as to their relation to the lime salts, which have been found to have zymoplastic activity by other investigators.

The constituents of the cells which hinder coagulation and which are insoluble in alcohol-ether are compound proteins, and have been called *cytoglobin* and *preglobulin* by SCHMIDT. The retarding action of these bodies may be suppressed by the addition of zymoplastic substances, and the yield of fibrin on coagulation in this case is much greater than in the absence of the compound protein retarding coagulation. This last supplies the material from which the fibrin is produced. The process is, according to SCHMIDT, as follows: The preglobulin first splits, yielding serglobulin, then from this the fibrinogen is derived, and from this latter the fibrin is produced. The object of the thrombin is twofold. The thrombin first splits the fibinogen from the paraglobulin and then converts the fibrinogen into fibrin. The assumption that fibrinogen can be split from paraglobulin has not sufficient foundation and is even improbable.

According to SCHMIDT the retarding action of the cells is prominent during life, while the accelerating action is especially pronounced outside of the body or by coming in contact with foreign bodies. The parenchymous masses of the organs and tissues, through which the blood flows in the capillaries, are those cell-masses which serve to keep the blood fluid during life.

LILIENFELD has given further proof as to the occurrence in the form-

elements of the blood of bodies which accelerate or retard the coagulation. According to this author the nature of these bodies is very markedly different from SCHMIDT's idea. While, according to SCHMIDT, the coagulation accelerators are bodies soluble in alcohol, and the compound proteins exhausted with alcohol act only retardingly on coagulation, LILIENFELD states that the substance which acts acceleratingly and retardingly on coagulation are contained in a nucleoprotein, namely, nucleohistone. Nucleohistone readily splits into leuconuclein and histone, the first of which acts as a coagulation-excitant, while the other, introduced into the blood-vascular system, either intravascular or extravascular, robs the blood of its property of coagulating. Introduced into the circulatory system the nucleohistone splits into its two components. It therefore causes extensive coagulation on one side and makes the remainder of the blood uncoagulable on the other. This theory as well as that of SCHMIDT is not based upon sufficiently demonstrated facts.

BRÜCKE showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation in liquids poor in ferment has been known for several years through the researches of HAMMARSTEN, GREEN, RINGER and SAINSBURY. The necessity of the lime salts for the coagulation of blood and plasma was first shown positively by the important investigations of ARTHUS and PAGÈS. Recent investigations of SABBATANI[1] have also shown the importance of calcium salts or the free calcium ions for coagulation without explaining the mode of their action.

According to the generally accepted view of ARTHUS and PAGÈS the soluble lime salts precipitable by oxalate are necessary requisites for the fermentive transformation of fibrinogen, because thrombin remains inactive in the absence of soluble lime salts. This view is untenable, as shown by the researches of ALEX. SCHMIDT, PEKELHARING, and HAMMARSTEN.[2] Thrombin acts as well in the absence as in the presence of precipitable lime salts.

LILIENFELD'S theory that the leuconuclein splits off a protein substance, *thrombosin*, from the fibrinogen, and that this thrombosin forms an insoluble compound with the lime present, producing thrombosin lime (fibrin), which separates, is incorrect according to HAMMARSTEN, SCHÄFER, and CRAMER.[3] LILIENFELD'S thrombosin is nothing but fibrinogen which is precipitated by a lime salt from a salt-poor or salt-free solution.

According to PEKELHARING[4] thrombin is the lime compound of

[1] Hammarsten, Nova Acta reg. Soc. Scient. Upsal. (3), **10**, 1879; Green, Journ. of Physiol., 8; Ringer and Sainsbury, *ibid.*, **11** and **12**; Arthus et Pagès and Arthus, see footnote 6, p. 243; Hammarsten, Zeitschr. f. physiol. Chem., **22**; Sabbatani, cited, Centralbl. f. Physiol., **16**, 665.

[2] Hammarsten, Zeitschr. f. physiol. Chem., **22**, where the other investigators are cited.

[3] Hammarsten, l. c.; Schäfer, Journ. of Physiol., **17**; Cramer. Zeitschr. f. physiol. Chem., **23**.

[4] See footnote 3, p. 248, and especially Virchow's Festschrift, **1**, 1891.

prothrombin, and the process of coagulation consists, according to him, in the thrombin transferring the lime to the fibrinogen, which is thereby converted into an insoluble lime compound, fibrin. Of the objections to this theory can be mentioned, among others, the fact that fibrin has not been obtained absolutely free from lime, but still so poor in lime (HAMMARSTEN [1]) that if the lime belongs to the fibrin, its molecule must be more than ten times greater than the hæmoglobin molecule, which is not probable. These as well as many other observations indicate that the lime is carried down by the fibrinogen only as a contamination.

If, as it seems, the lime is not of importance in the transformation of fibrinogen into fibrin in the presence of thrombin, still this does not contradict the above-mentioned observations of ARTHUS and PAGÈS that the lime salts are necessary for coagulation of blood and plasma. It is very probable that the lime salts, as admitted by PEKELHARING, are a requisite for the transformation of prothrombin into thrombin.

If we attempt to summarize the more or less contradictory investigations and views as given in the preceding pages, we can consider the following facts as conclusive: In the first place, two bodies, the fibrinogen and the thrombin, are necessary for the coagulation. The fibrinogen exists preformed in the plasma. The thrombin, on the contrary, does not occur in living blood, at least not in appreciable amounts as such, but is formed from another substance, the prothrombin. The presence of calcium salts is necessary for the formation of this thrombin, while the calcium salts are not necessary for the enzymotic transformation of fibrinogen into fibrin. Besides the calcium salts also other substances, the zymoplastic active substances, are active in the formation of thrombin from its mother-substance, and these zymoplastic substances stand in some relation to the form-elements of the blood.

The formation of thrombin and the relation of the form-elements therewith are still unexplained or disputed questions.

It is a question whether the mother-substance of thrombin exists in the plasma of the circulating blood or whether it is a body eliminated from the form-elements before coagulation. We have two opposing views on this question, namely, those of ALEX. SCHMIDT and of PEKELHARING. According to SCHMIDT prothrombin occurs preformed in the circulating plasma, and it is transformed into thrombin by the zymoplastic substances which pass out from the form-elements. PEKELHARING, on the contrary, holds the view that the plasma does not contain appreciable amounts of prothrombin. This body, according to him, passes before coagulation from the form-elements into the plasma, and

[1] Zeitschr. f. physiol. Chem., 28.

is there converted into thrombin by the calcium salts. The observation that uncoagulated leech-plasma does not coagulate on the addition of calcium salts, while it does coagulate on the addition of prothrombin solutions, seems to support this view; still it is not quite conclusive. Leech-extract contains a body, hirudin, which, according to MORAWITZ, is an antibody toward thrombin and quantitatively neutralizes it. On the addition of prothrombin, new thrombin may be formed, which may act if the hirudin is not present in too great an excess.

The behavior of sodium-fluoride plasma shows more conclusively the absence of prothrombin in the circulating plasma. Such plasma, according to ARTHUS, contains no prothrombin, a statement which has been partly substantiated by MORAWITZ, who finds that fluoride-plasma contains more or less prothrombin, dependent upon the greater or less change the blood undergoes before it flows into the sodium-fluoride solution. One can obtain, according to MORAWITZ, at least sometimes, a fluoride-plasma which contains no prothrombin. The observations of FULD and of SCHITTENHELM and BODONG contradict the statement that fluoride-plasma contains prothrombin. As BORDET and GENGOU[1] have shown that prothrombin can be carried down by the precipitate produced in fluoride-plasma, it seems as if the observations of ARTHUS and MORAWITZ on this point are not conclusive, and MORAWITZ is now also of the opinion that the prothrombin occurs preformed in the plasma. NOLF[2] also holds this view, and for the present we generally believe that prothrombin, or as it is also now designated *thrombogen* (MORAWITZ, NOLF), is a preformed constituent of the plasma. The absence of prothrombin, as observed by ARTHUS, in peritoneal transudates in the horse, can hardly be considered as sufficient evidence as to the non-occurrence of this body in blood-plasma.

Although the opinions are rather united as to the occurrence of at least three bodies, fibrinogen, prothrombin (thrombogen) and lime salts in the plasma, still the question arises how the thrombin is formed from the thrombogen. The zymoplastic substances must be here considered, and the starting-point in these new investigations is the accelerating action upon coagulation, of different tissue extracts, an action which has been known for a long time and was especially studied by DELEZENNE on the plasma from bird's blood. Unfortunately we are not in accord

[1] Arthus, Journ. de Physiol. et Pathol., **3** and **4**, and Compt. rend. soc. biol., **56**. The works of Morawitz may be found in Hofmeister's Beiträge, **4** and **5**, Deutsch. Arch. f. klin. Med., **79** and **80**, and in Oppenheimer's Handb der Bioch., **2**; Fuld, Centralbl, f. Physiol., **17**, p. 529; with Spiro, Hofmeister's Beiträge, **5**; Schittenhelm and Bodong, Arch. f. exp. Path. u. Pharm., **54**; Bordet and Gengou, Annal. Institut Pasteur, **18**. For more recent literature see Loeb, Biochem. Centralbl., **6**, p. 907.

[2] Arch. internat. de Physiol., **6**, 1908.

as to the nature and manner of action of the active constituents of these extracts. According to MORAWITZ the active body is not thrombin, but another substance called *thrombokinase*, besides lime-salts, which are necessary for the transformation of prothrombin (*thrombogen* according to MORAWITZ). The production of thrombokinase is, according to MORAWITZ, a general property of the protoplasm, and also occurs in the leucocytes (and blood-plates). Three substances are necessary, according to his view, for the formation of thrombin, namely: Thrombogen, thrombokinase and lime salts. Thrombogen is, he claims, not quite identical with the prothrombin (other investigators), which he calls α-prothrombin, but is a mother-substance of it. The process of thrombin formation can be given as follows: The kinase first transforms the thrombogen into α-prothrombin, which latter then is converted into thrombin (α) by the lime-salts.

The thrombokinase also does not occur to any appreciable extent in the circulating blood, but is supplied by the form-elements. The accelerating action upon coagulation of tissues or parts of tissues depends, as above stated, upon their content of kinase; but it also in part depends upon the fact that the tissue fluids excite the secretory activity of the form-elements.

FULD [1] has arrived at about the same results independently of MORAWITZ, but he has selected other names. The three substances thrombogen, kinase, and thrombin are called by him *plasmozym*, *cytozym*, and *holozym*. The chief reason why circulating blood remains fluid is, according to FULD, because the cytozym is only slowly formed therein and the ferment (holozym) produced thereby is quickly changed into an inactive form. Another reason is that the blood contains an antibody for the fibrin ferment. The assumption of ALEXANDER SCHMIDT that the blood contains substances retarding coagulation (anti-thrombins) has recently also received support by the observations of FULD and SPIRO, MORAWITZ, LOEB, NOLF, PUGLIESE [2] and others.

According to the theory of MORAWITZ, FULD and SPIRO, of those substances necessary for coagulation, only the thrombokinase (the cytozym) is absent in the circulating blood, and this is the reason why the circulating blood remains fluid. The reason why the plasma does not contain any thrombokinase lies in the fact that the healthy endothelium of the vessels does not have any irritating action upon the form-elements, and therefore no mentionable quantity of kinase is given off under these circumstances. Such an elimination occurs first outside of the blood vessels, and indeed very quickly in contact with foreign bodies. The formation of thrombin from the thrombogen takes place in an unknown manner by the action of the kinase only in the presence of lime salts (in the plasma), and this thrombin then transforms the fibrinogen into fibrin.

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[1] Centralbl. f. Physiol., **17**. See also Fuld and Spiro, Hofmeister's Beiträge, **5**.

[2] Fuld and Spiro, l. c.; Morawitz, l. c.; Loeb, Hofmeister's Beiträge, **5**; Nolf, Arch. internat. de Physiol., **6**; Pugliese, Biochem. Centralbl., **5**, p. 930.

A serum poor in ferment and having a weak action can be reactivated by the addition of acid or alkali (ALEX. SCHMIDT, MORAWITZ), and in this action, according to MORAWITZ, a thrombin (β) is produced which is somewhat different from α-thrombin. The β-thrombin is produced from a special β-prothrombin which never occurs in the plasma, but only in the serum. FULD explains this by affirming that the α-thrombin is changed in the serum into *metazym* (β-prothrombin), which is then transformed by the alkali or acid into *neozym* ($=\beta$-thrombin). Nevertheless it is a fact that the quantity of thrombin in the serum diminishes after coagulation and that the thrombin action is considerably increased by the addition of alkali or acid as well as by zymoplastic substances. The above view as to the occurrence of different thrombins has not sufficient basis, and PEKELHARING [1] has also raised objections thereto.

The theories of MORAWITZ, FULD and SPIRO at least stand in accord with several known facts but do not take sufficient account of the action of the zymoplastic substances of ALEX. SCHMIDT. Thrombokinase is precipitated by alcohol and is not thermostabile, while the zymoplastic substances, of SCHMIDT are thermostabile and soluble in alcohol. The thrombokinase cannot therefore be identical with these zymoplastic substances, and hence this theory does not explain the action of these latter. Further, the mode of action of tissue extracts is unexplained, and is a much disputed subject. It can be said that these two views are in the main opposed to each other. According to one (ALEX. SCHMIDT, ARTHUS, MORAWITZ and others) they do not act like fibrin ferment, but have an indirect action. According to the other (PEKELHARING, HUISKAMP, DELEZENNE and LOEB [2]) they are thrombin, or at least bodies having an analogous action.

L. LOEB,[3] who has carried out complete investigations on the coagulation of blood, especially of Crustaceæ, has arrived at the following view: The coagulation in the Crustaceæ can, according to him, be of two kinds. It may in part be an agglutination of the amœbocytes and in part a fibrin formation from a fibrinogen of the plasma. This latter coagulation is essentially the same as occurs in vertebrates. The substance acting here as the excitant for the coagulation is also active in the absence of lime salts, and behaves therefore like a thrombin. The tissues contain constituents which accelerate coagulation which LOEB calls *coagulins*, which are not identical with the coagulins of the clot or the blood serum, and these have also, although only in the presence of lime salts (if the author understands LOEB), a direct coagulating action upon fibrinogen. According to LOEB the tissue coagulins do not act as kinases in the invertebrates, and he also finds it improbable that

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[1] Bioch. Zeitschr., **11**.

[2] Huiskamp. Zeitschr. f. physiol. Chem., **34**, **39**; Delezenne, Arch. de physiol., 1897; Loeb, Biochem. Centralbl., **6**, pages 829 and 889.

[3] Medical News, New York, 1903, and Virchow's Arch., **176**; Hofmeister's Beiträge, **5**, **6**, **8**, **9**, and Biochem. Centralbl., **6**, pages 829 and 889.

they would act as kinases in the vertebrates. Under favorable conditions the combined blood and tissue coagulins are more active than the sum of the individual action. That this is due to an activation by a kinase, which is a possible explanation, is, in LOEB'S opinion, not proven.

The coagulins of the blood are, as above stated, according to LOEB, different from the tissue coagulins. The first show no specific action, i.e., between invertebrates and vertebrates. The tissue coagulins, on the contrary, have by their action upon the blood a certain specificity, at least in animals widely separated from one another.

Opinions are strikingly at variance in regard to the mode of action of the tissue constituents which accelerate coagulation, and their nature also is entirely unknown, hence great confusion exists on the whole in this subject.

If we accept the fact that thrombokinase does not occur in the plasma, but is produced under the influence of a foreign body acting as an excitant, it is rather difficult to understand why the plasma obtained from blood collected in a paraffined vessel and quickly and strongly centrifuged, and which is perfectly free from form-elements, should remain fluid for a long time in a paraffined vessel while it coagulates in an ordinary glass vessel. NOLF has tried by his theory to explain this difficulty, as well as the action of the alcohol-soluble zymoplastic substances (ALEX. SCHMIDT).

According to NOLF[1] the following bodies take direct part in the coagulation of the blood, namely: *Fibrinogen, thrombogen* (formerly called hepatothrombin by him) *thrombozym* (=thrombokinase of MORAWITZ) and *lime salts.* The coagulation of the blood, according to him, is a different process from the coagulation of a fibrinogen solution by thrombin. While in this last case the thrombin is the substance exciting coagulation, in the other case the thrombin is a product of the coagulation, as suggested by WOOLDRIDGE. In the coagulation of the plasma, according to NOLF we have a mutual precipitation of the three above-mentioned colloids—fibrinogen, thrombogen and thrombozym, all three of which are contained in the fibrin clot. This latter has correspondingly no constant composition, but varies according to the relative proportions of these three colloids. In the presence of only a little fibrinogen thrombin is produced from the three colloids (in the presence of lime salts); in the presence of abundance of fibrinogen on the contrary fibrin is formed. Thrombin is a fibrin incompletely saturated with fibrinogen, and in the coagulation of fibrinogen with thrombin the still unsatisfied affinities of the latter are saturated (" La thrombine d'A. Schmidt n'est pas autre chose que la fibrine insuffisamment pourvue de fibrinogène. Dans la

[1] Arch. internat. de Physiol., 6, Fasc., 1, 2 and 3.

coagulation du fibrinogène par la thrombine les affinités restées libres de celle-ci peuvent s'assouvir; le composé moins saturé se transforme en un composé plus saturé.") The formation of fibrin from fibrinogen is not, according to NOLF, an enzymotic process.

In NOLF's opinion the thrombogen is probably formed in the liver and found to a large extent in all plasma. The thrombozym is secreted by the leucocytes and the endothelial cells. It is also a normal constituent of the blood plasma circulating in the living body. Most tissues, on the contrary, contain no thrombozym. The tissue extracts, NOLF believes, also contain no substances absolutely necessary for the coagulation, but only bodies which can have a powerful accelerating action, the *thromboplastic* substances. The circulating blood plasma contains all the bodies directly necessary in the coagulation, namely, fibrinogen, thrombogen, thrombozym and lime salts. Besides these it also contains a substance that inhibits coagulation, *antithrombin*, which is formed in the liver and which NOLF now considers as a special substance and not as he formerly believed, an excess of thrombogen. There exists, if the author understands the work of NOLF, a labile equilibrium between the various constituents of the plasma, and this equilibrium is destroyed in coagulation. The first impulse to coagulation is given by the thromboplastic substances.

NOLF considers as thromboplastic active any influence of a physical or chemical nature which, be it produced by the walls of the vessel, a suspended body, a solvent or a dissolved body, a colloid or crystalloid, a molecule or an ion, makes the combination of the three above colloids possible. To the thromboplastic agents belong the walls of a glass vessel, finely powdered glass, the precipitates of calcium oxalate or calcium fluoride, also living protoplasm, aqueous tissue extracts, the alcohol soluble zymoplastic substances of ALEX. SCHMIDT, and other substances. All these agents in some way or other may serve as points of precipitation; but unfortunately it is not clear how this thromboplastic action is brought about.

An important side of NOLF's theory of coagulation is also the *fibrinolysis* which is brought about by the thrombin. The proteolytic action of the thrombin is due only to the thrombozym contained therein, and it has a proteolytic action only upon fibrin and not upon fibrinogen. According to NOLF, coagulation is merely a preparation for the proteolysis, and is a nutrition phenomenon, and in addition is of special importance, in arresting hemorrhage. In order to prevent a rapid fibrinoylsis, the plasma also contains one or more antifibrinolytic substances, which are secreted by the liver.

What has been given contains the chief points in NOLF's theory of coagulation, and it is impossible in a text-book to enter more into detail

in regard to his remarkable investigations or the foundations on which he bases his theory and the objections which can be raised against it.

From the above description of the various theories of coagulation it at least follows that in the study of the coagulation of the blood there are many contradictory statements and observations, and so many obscure points, that for the present it is impossible to give a clear, comprehensive summary of the different views and to deduce a theory of the process of coagulation which would embrace all the factors.

In spite of this confusion and all contradictions, still we are sure that certain bodies such as fibrinogen and thrombin, even though this latter be an enzyme or a colloid combination, are directly concerned in the formation of fibrin, while other bodies act indirectly as accelerators or inhibitors of coagulation.

The bodies accelerating coagulation, with the exception of gelatin, whose action in this regard has not been positively proven, have been mentioned several times above. The mode of action of the bodies retarding coagulation is not clear and is much disputed. Their action may, it seems, also be more of a direct or indirect kind. Thus, for example, the oxalate and fluoride may prevent the formation of thrombin by precipitation of the lime. The cobra-poison seems to prevent the formation of thrombin by the action upon the thrombokinase; the hirudin [1] may, it is generally believed, as antithrombin make the thrombin inactive, and the normal constituents of the plasma retarding coagulation perhaps act in a similar manner. In other cases the retarding bodies act indirectly, for they may, like the proteoses and others, cause the body to produce special bodies which stand in close relation to intravascular coagulation.

Intravascular Coagulation. It has been shown by Alex. Schmidt and his students, as also by Wooldridge, Wright,[2] and others, that an intravascular coagulation may be brought about by the intravenous injection into the circulating blood of a large quantity of a thrombin solution, as also by the injection of leucocytes or tissue fibrinogen (impure nucleoprotein). Intravascular coagulation may also be brought about under other conditions, such as after the injection of snake-poison (Martin [3] and others) or certain of the protein-like colloid substances, synthetically prepared according to Grimaux's method (Halliburton and Pickering [4]). If too little of the above-mentioned bodies be injected, then we observe only a marked retarding tendency in the coagulation of the

[1] The action of hirudin is somewhat doubtful. See Schittenhelm and Bodong, l. c.

[2] A Study of the Intravascular Coagulation, etc., Proceed. of the Roy. Irish Acad. (3), 2. See also Wright, Lecture on Tissue or Cell Fibrinogen, The Lancet, 1892; and Wooldridge's Method of Producing Immunity, etc., Brit. Med. Journ., Sept., 1891.

[3] Journ. of Physiol., 15.

[4] *Ibid.*, 18.

blood. According to WOOLDRIDGE it can generally be maintained that after a short stage of accelerated coagulability, which may lead to a total or partial intravascular coagulation, a second stage of a diminished or even arrested coagulability of the blood follows. The first stage is designated (WOOLDRIDGE) as the *positive* and the other as the *negative phase* of coagulation. These statements have been confirmed by several investigators.

There is no doubt that the positive phase is brought about by an abundant introduction of thrombin, or by a rapid and abundant formation of the same. The explanation of the production of the negative phase, which can easily be brought about by pepsin proteoses, by various bodies such as extracts of crabs' muscles and other organs, eel-serum, enzymes, bacterial toxines, certain snake-poisons, etc., has been attempted in different ways. The best studied is the action of proteoses, but no conclusive results have been obtained thus far. The assertion of PICK and SPIRO that the action of the proteoses does not depend upon the proteoses themselves, but upon a contaminating substance, the *protozym*, is claimed to be incorrect by UNDERHILL, while the recent investigations of POPIELSKI indicate that this is correct. The bodies retarding coagulation obtained by CONRADI [1] in autolysis, which are probably antithrombins, seem to act in a different way from the proteoses, and cannot for the present be made use of in explaining this question.

There are a large number of researches on the action of proteoses and of other retarding substances by different investigators, such as GROSJEAN, LEDOUX, CONTEJEAN, DASTRE, FLORESCO, ATHANASIU, CARWALLO, GLEY, PACHON and GLEY, SPIRO and ELLINGER, FULD and SPIRO, MORAWITZ and NOLF, but those of DELEZENNE [2] are of the greatest importance. We can say with certainty that the action is indirect and that the liver is important for the process. The non-coagulability of "peptone-blood" seems to be due to several reasons, but it has not been thoroughly explained. On the one hand such blood contains an antithrombin and on the other it seems as if the formation of thrombin is not sufficient, although the plasma contains the necessary conditions for the thrombin formation, as it coagulates as a rule on dilution with

[1] Pick and Spiro, Zeitschr. f. physiol. Chem., **31**; Underhill, Amer. Journ. of Physiol., **9**; Popielski, Arch. f. expt. Path. u. Pharm. Suppl., 1908, Schmiedeberg's Festschrift; Conradi, Hofmeister's Beiträge, **1**.

[2] Grosjean, Travaux du laboratoire de L. Fredericq, **4**, Liège, 1892; Ledoux, *ibid.*, **5**, 1896; Nolf, Bull. l'Acad. roy. de Belgique, 1902 and 1905, and Biochem. Centralbl., **3**; Spiro and Ellinger, Zeitschr. f. physiol. Chem., **23**; Fuld and Spiro, l. c.; Morawitz, l. c. The works of the above-mentioned French investigators can be found in Compt. rend. soc. biol., **46**, **47**, **48**, **50**, and **51**, and Arch. d. Physiol. (5), **7**, **8**, **9**, and **10**; see also especially Delezenne, Arch. d. Physiol. (5), **10**; Compt. rend. soc. biol., **51**, and Compt. rend., **130**.

water or the addition of a little acid. Opinions in regard to the occurrence of an antithrombin in the peptone-plasma seem to be unanimous, and we have collected rather considerable experience in regard to the formation of this antithrombin. According to NOLF, the peptone (more correctly the proteoses) causes an alteration in the leucocytes and the walls of the vessels, and a substance is secreted which brings about, in the liver, the formation of antithrombin. According to DELEZENNE the proteoses bring about a destruction of leucocytes, and thereby a substance accelerating coagulation and another having a retarding action are set free. The first is destroyed by the liver, and hence the action of the retarding substance (the antithrombin) is obtained. The only thing that is positively proven is the part taken by the liver in this retardation of coagulation, as shown by GLEY and PACHON; the non-appearance of the thrombin formation is not explained by the above theories.

The reason of the slow coagulation of the blood in *hæmophilia* is not well known. Recent investigations of MORAWITZ and LOSSEN [1] seem to show that a lack of thrombokinase plays an important part in this condition. This explains the repeatedly observed relation of the vessel-walls to hæmophilia as, according to NOLF, the thrombokinase (his thrombozym) is also secreted by the endothelial cells.

The non-coagulability of cadaver blood depends usually, according to MORAWITZ,[2] upon the fact that it contains no fibrinogen, due to a fibrinolysis.

The *gases of the blood* will be treated of in Chapter XVII (on respiration).

IV. THE QUANTITATIVE COMPOSITION OF THE BLOOD.

The quantitative analyses of the blood are of little value. We must ascertain on one side the relation of the plasma and blood-corpuscles to each other, and on the other the constitution of each of these two chief constituents. The difficulties which stand in the way of such a task, especially in regard to the living, non-coagulated blood, have not been removed. Since the constitution of the blood may differ not only in different vascular regions, but also in the same region under different circumstances, which renders a number of blood analyses necessary, it can hardly appear remarkable that our knowledge of the constitution of the blood is still relatively limited.

The relative volume of blood-corpuscles and serum in blood has been determined by various methods. Of these methods that of L. and M. BLEIBTREU [3] against which important objections have been raised by

[1] Deutsch. Arch. f. klin. Med., **94**.
[2] Hofmeister's Beiträge, 8.
[3] Pflüger's Arch., **51**, **55**, and **60**.

several investigators, such as EYKMAN, BIERNACKI and HEDIN,[1] must be especially mentioned. In regard to this as well as to the method of St. BUGARSKY and TANGL, which is based upon a difference in the electrical conductivity of the blood and the plasma, and STEWART'S[2] colorimetric method, we must refer to the original publications.

For clinical purposes the relative volume of corpuscles in the blood may be determined by the use of a small centrifuge called a *hæmatocrit*, constructed by BLIX and described and tested by HEDIN. A measured quantity of blood is mixed with a known volume (best an equal volume) of a fluid which prevents coagulation. This mixture is introduced into a tube and then centrifuged. According to HEDIN it is best to treat the blood, which is kept fluid by 1 p. m. oxalate, with an equal volume of a 9 p. m. NaCl solution. After complete centrifugalization, the layer of blood-corpuscles is read off on the graduated tube and the volume of blood-corpuscles (or more correctly the layer of blood-corpuscles) in 100 vols. of the blood calculated therefrom. By means of comparative counts, HEDIN and DALAND have found that an approximately constant relation exists between the volume of the layer of blood-corpuscles and the number of red corpuscles under physiological conditions, so that the number of corpuscles may be calculated from the volume. DALAND[3] has shown that such a calculation gives approximate results also in disease, when the size of the blood-corpuscles does not essentially deviate from the normal. In certain diseases, such as pernicious anæmia, this method gives such inaccurate results that it cannot be used.

KÖPPE[4] has shown that in centrifuging blood very rapidly, more than 5000 times per minute, the blood-corpuscles may be so completely separated that all intermediate fluid is removed. Because of the absence of this intermediate fluid the refraction is changed; the outer layers of the erythrocytes containing fat become transparent, and the column of blood-corpuscles becomes transparent and laky. If the volume of the separated column of blood-corpuscles is determined and the number of red blood-corpuscles counted, the absolute volume of these latter can be determined by this method.

In determining the relation between the weight of blood-corpuscles and the weight of blood-fluid, we generally proceed in the following manner:

If any substance is found in the blood which belongs exclusively to the plasma and does not occur in the blood-corpuscles, then the amount of plasma contained in the blood may be calculated if we determine the amount of this substance in 100 parts of the plasma or serum respectively on the one side, and in 100 parts of the blood on the other. If we represent the amount of this substance in the plasma by p and that in the blood by b, then the amount of x in the plasma from 100 parts of blood is is $x = \frac{100.b}{p}$.

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[1] Biernacki, Zeitschr. f. physiol. Chem., **19**; Eykman, Pflüger's Arch., **60**; Hedin, *ibid.*, and Skand. Arch. f. Physiol., **5**.

[2] Bugarsky and Tangl, Centralbl. f. Physiol., **11**; Stewart, Journ. of Physiol., **24**.

[3] Hedin, Skand. Arch. f. Physiol., **2**, 134 and 361, and **5**; Pflüger's Arch., **60**; Daland, Fortschritte d. Med., **9**.

[4] Pflüger's Arch., **107**.

Such a substance, which occurs only in the plasma, is fibrin according to HOPPE-SEYLER, sodium according to BUNGE (in certain kinds of blood), and sugar according to OTTO.[1] The experimenters just named have tried to determine the amount of the plasma and blood-corpuscles, respectively, in different kinds of blood, starting from the above-mentioned substances.

Another method suggested by HOPPE-SEYLER is to determine the total amount of hæmoglobin and proteins in a portion of blood, and on the other hand the amount of hæmoglobin and proteins in the blood-corpuscles (from an equal portion of the same blood) which have been sufficiently washed with common-salt solution by centrifugal force. The figure obtained as a difference between these two determinations corresponds to the amount of proteins which was contained in the serum of the first portion of blood. If we now determine the proteins in a special portion of serum of the same blood, then the amount of serum in the blood is easily determined. The usefulness of this method has been confirmed by BUNGE by the control experiments with sodium determinations. If the amount of serum and blood-corpuscles in the blood is known, and we then determine the amount of the different blood-constituents in the blood-serum on one side and of the total blood on the other, the distribution of these different blood-constituents in the two chief components of the blood, plasma and blood-corpuscles may be ascertained. In the table opposite are given analyses of the blood of various animals by ABDERHALDEN[2] according to HOPPE-SEYLER'S and BUNGE'S methods. The analyses of human blood by C. SCHMIDT[3] are older and were made according to another method, hence the results for the weights of the corpuscles are perhaps a little too high. All the results are in parts per 1000 parts of blood.

The relation between blood-corpuscles and plasma may vary considerably under different circumstances even in the same species of animal. In animals, in most cases considerably more plasma is found, sometimes two-thirds of the weight of the blood.[4] For human blood ARRONET has found 478.8 p. m. blood-corpuscles and 521.2 p. m. serum (in defibrinated blood) as an average of nine determinations. SCHNEIDER[5] found 349.6 and 650.4 p. m. respectively in women.

The sugar was considered as occurring only in the serum and not with the blood-corpuscles. According to the recent investigations of RONA and MICHAELIS the blood-corpuscles of the dog contain considerable amounts of sugar; and the quantity of sugar in the blood, in the blood-cor-

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[1] Hoppe-Seyler, Handb. d. physiol. u. path. chem. Analyse, 6. Aufl.; Bunge, Zeitschr. f. Biologie, **12**; Otto, Pflüger's Arch., **35**.

[2] Zeitschr. f. physiol. Chem., **23** and **25**.

[3] Cited and in part recalculated from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., 345.

[4] See Sacharjin in Hoppe-Seyler's Physiol. Chem., 447; Otto, Pflüger's Arch., **35**; Bunge, l. c.; L. and M. Bleibtreu, Pflüger's Arch., **51**.

[5] Arronet, Maly's Jahresber., **17**; Schneider, Centralbl. f. Physiol., **5**, 362.

	Pig-blood.		Ox-blood.		Horse-blood.		Dog-blood.		Bull-blood.		Sheep-blood.	
	Blood-corpuscles, 435.09.	Serum, 564.91.	Blood-corpuscles, 325.5.	Serum, 674.5.	Blood-corpuscles, 397.7.	Serum, 602.3.	Blood-corpuscles, 442.8.	Serum, 577.2.	Blood-corpuscles, 334.3.	Serum, 665.7.	Blood-corpuscles, 319.2.	Serum, 680.8.
Water	272.20	518.36	192.65	616.25	243.87	551.14	277.71	514.30	206.81	608.03	200.03	624.16
Solids	162.89	46.54	132.85	58.249	153.84	51.15	165.10	42.89	127.50	57.66	118.82	56.63
Hæmoglobin	142.20	—	103.10	—	125.8	—	145.6	—	106.40	—	102.80	—
Protein	8.35	38.26	20.89	48.901	20.05	42.65	2.36	34.05	15.38	46.41	12.80	46.56
Sugar	—	0.684	—	0.708	—	0.90	—	0.74	—	0.679	—	0.708
Cholesterin	0.213	0.231	1.100	0.835	0.26	0.31	0.56	0.37	0.610	0.599	1.147	0.891
Lecithin	1.504	0.805	1.220	1.129	1.93	1.05	1.02	0.98	0.953	1.244	1.329	1.088
Fat	—	1.104	—	0.625	—	0.50	—	0.91	—	2.357	—	0.859
Fatty acids	0.027	0.448	—	—	0.02	0.36	—	0.70	—	0.494	—	0.4908
Phosphoric acid as nuclein	0.0455	0.0123	0.0178	0.0089	0.05	0.01	0.05	0.01	0.0194	0.0089	0.0235	0.0109
Soda	—	2.401	0.7266	2.9084	—	2.62	1.27	2.39	0.839	2.873	0.760	2.917
Potash	2.157	0.152	0.2351	0.1719	1.32	0.15	0.11	0.14	0.233	0.174	0.236	0.172
Iron oxide	0.696	—	0.544	—	0.59	—	0.71	—	0.562	—	0.545	—
Lime	—	0.0689	—	0.0805	—	0.07	—	0.06	—	0.073	—	0.089
Magnesia	0.0656	0.0233	0.0056	0.0300	0.04	0.03	0.03	0.03	0.009	0.027	0.006	0.027
Chlorine	0.642	2.048	0.5901	2.4889	0.18	2.20	0.60	2.31	0.628	2.453	0.575	2.516
Phosphoric acid	0.8956	0.1114	0.2392	0.1646	0.98	0.15	0.67	0.14	0.236	0.156	0.228	0.163
Inorganic P_2O_5	0.7194	0.0296	0.1140	0.0571	0.76	0.05	0.54	0.05	0.133	0.041	0.088	0.057

	Goat-blood.		Cat-blood.		Rabbit-blood.		Human Blood, Man.		Human Blood, Woman.	
	Blood-corpuscles, 347.2.	Serum, 652.8.	Blood-corpuscles, 434.0.	Serum, 566.0.	Blood-corpuscles, 372.1.	Serum, 627.9.	Blood-corpuscles, 513.02.	Serum, 486.98.	Blood-corpuscles, 396.24.	Serum, 603.76.
Water	211.35	592.54	270.90	524.17	235.74	518.18	349.69	439.02	272.56	551.99
Solids	135.86	60.25	163.11	41.35	136.37	46.71	163.33	47.96	123.68	51.77
Hæmoglobin	112.50	—	143.2	—	123.50	—				
Protein	18.76	50.96	11.62	33.16	4.55	33.63				
Sugar	—	0.822	—	0.860	—	1.036	Organic bodies			
Cholesterin	0.601	0.698	0.556	0.339	0.268	0.343				
Lecithin	1.339	1.127	1.354	0.971	1.722	1.105	159.59	43.82	120.13	46.70
Fat	—	0.0407	—	0.446	—	0.749				
Fatty acids	—	0.398	—	0.282	—	0.507				
Phosphoric acid as nuclein	0.028	0.0117	0.063	0.009	0.040	0.015	Inorg. 3.74	4.14	3.55	5.07
Soda	0.755	2.824	1.174	2.512	—	2.789	0.24	1.66	0.65	1.92
Potash	0.236	0.160	0.112	0.148	1.946	0.162	1.59	0.15	1.41	20.20
Iron oxide	0.547	—	0.694	—	0.615	—	—	—	—	—
Lime	—	0.078	—	0.062	—	0.072	—	—	—	—
Magnesia	0.014	0.026	0.035	0.024	0.029	0 028	—	—	—	—
Chlorine	0.514	2.409	0.455	2.360	0.460	2.438	0.90	1.72	0.36	0.14
Phosphoric acid	0.243	0.154	0.697	0.133	0.835	0.151	—	—	—	—
Inorganic P_2O_5	0.097	0.045	0.515	0.040	0.645	0.040	—	—	—	—

puscles as well as in the plasma, of man with diabetes mellitus is increased. They found the same in dogs with alimentary glycosuria.[1] HOLLINGER[2] also found that in man with normal quantity of sugar in the blood the sugar was distributed nearly equally between the blood-corpuscles and the plasma, and in most of the cases of hyperglycæmia investigated the blood-corpuscles contained much more of the

[1] Biochem. Zeitschr., **16** and **18**.

[2] Biochm. Zeitschr., **18**.

abnormally high sugar content than the serum. As the blood-corpuscles of drawn blood are impermeable to sugar, a fact further substantiated by special experiments by MICHAELIS and RONA, the reason for the permeability of the corpuscles in life must be investigated. According to ABDERHALDEN lime, fat and perhaps also fatty acids occur only in the serum. The small traces of bile-acids found in normal blood are, according to CROFTAN,[1] contained in the leucocytes. The division of the alkalies between the blood-corpuscles and the plasma is different, as the blood-corpuscles from the pig, horse, and rabbit contain no soda, those from human blood are richer in potassium, and the corpuscles from ox-, sheep-, goat-, dog-, and cat-blood are considerably richer in sodium than potassium. Chlorine exists in all blood to a greater extent in the serum than in the blood-corpuscles. Iodine is found only in the serum, while iron occurs chiefly in the form-elements, especially in the erythrocytes. As the nucleoproteins contain iron, some iron always occurs in the leucocytes, and traces of iron are also found in the serum. This amount under normal conditions is very small, while in disease the relation between hæmoglobin-iron and other blood-iron does not seem to change very much. There are also found in the blood manganese and traces of lithium, copper, lead, silver, and in menstrual blood arsenic has also been noted. The blood as a whole contains in ordinary cases 770–820 p. m. water, with 180–230 p. m. solids; of these 173–220 p. m. are organic and 6–10 p. m. inorganic. The organic consists, deducting 6–12 p. m. of extractive bodies, of proteins and hæmoglobin. The amount of this last-mentioned body in human blood is about 130–150 p. m. In the dog, cat, pig, and horse the quantity of hæmoglobin is about the same, but is lower in the blood from the ox, bull, sheep, goat, and rabbit (ABDERHALDEN).

The amount of sugar in the blood is, according to most statements, on an average 1 p. m. It seems to be independent of the composition of the food, but feeding with large amounts of sugar or dextrin causes a considerable increase in the sugar of the blood, as observed by BLEILE. This condition does not seem to be the same for all animals, as shown by OPPLER and RONA; the amount of blood-sugar varied quite considerably in rabbits, while on the contrary in dogs it was very constant even under various conditions of life. When the quantity of sugar amounts to more than 3 p. m., then, according to CL. BERNARD,[2] sugar occurs in the urine and a glycosuria appears. An increase in the quantity of sugar takes place, as first observed by BERNARD and substantiated by FR. SCHENCK,

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[1] Pflüger's Arch., **90**.

[2] Bleile, Arch. f. (Anat. u.) Physiol., 1879; Oppler and Rona, Bioch. Zeitschr., **13**; Bernard, Leçons sur le diabète, Paris, 1877.

after removal of blood. According to HENRIQUES [1] this increase of the reducing power, at least in dogs, is not due to sugar, but chiefly to jecorin, which substance in normal blood is the cause of more of the reduction than the sugar. It is difficult to judge of the value of many reports as to the amount of sugar and the reducing power of the blood, because the experimenters generally have not considered the presence of a certain quantity of jecorin or conjugated glucuronic acids, or they were unable to detect them. The investigations of ANDERSSON [2] seem to indicate that at least in rabbits, on drawing blood, the fermentable sugar, as well as the non-fermentable "sugar rest," increases to about the same extent.[3]

The quantity of urea, which, according to SCHÖNDORFF, is equally divided between the blood-corpuscles and the plasma, is greater on taking food than in starvation (GRÉHANT and QUINQUAUD, SCHÖNDORFF) and varies between 0.2 and 1.5 p. m. In dogs SCHÖNDORFF found in starvation a minimum of 0.348 p. m. and a maximum of 1.529 p. m. at the point of highest urea formation. GOTTLIEB obtained much lower results by another direct method, namely, in starvation 0.1–0.2, and after meat feeding 0.28–0.56 p. m. In man v. JAKSCH [4] found 0.5–0.6 p. m. urea in normal blood. The quantity of urea is somewhat increased in fever, and in general in augmented protein metabolism the increased urea formation is dependent thereon. A more important increase in the quantity of urea in the blood occurs in a retarded elimination of urea, as in cholera, also in cholera infantum and in infections of the kidneys and urinary passages. After ligaturing the ureters or after extirpation of the kidneys of animals, an accumulation of urea takes place in the blood.

v. SCHRÖDER first showed that the blood of the shark was very rich in urea, and the quantity indeed amounted to 26 p. m. BAGLIONI [5] has recently shown that this large quantity of urea is of the greatest importance, as the presence of urea in these animals is a necessary life-condition for the heart and very probably for all organs and tissues.

The blood also contains traces of ammonia. According to HORODYNSKI, SALASKIN, and ZALESKI,[6] who worked with the improved NENCKI and ZALESKI method, the quantity in arterial dog-blood was 0.41 milli-

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[1] Schenck, Pflüger's Arch., **57**; Henriques, Zeitschr. f. physiol. Chem., **23**. See also Kolisch and Stejskal, Wien. klin. Wochenschr., 1898.

[2] Bioch. Zeitschr., **12**.

[3] In regard to the recent methods for determining sugar in the blood see Bang, Bioch. Zeitschr., **7**; Michaelis and Rona, *ibid.*, **7**; Oppler and Rona, *ibid.*, **13**.

[4] Gréhant et Quinquaud, Journ. de l'anatomie et de la physiol., **20**, and Compt. rend., **98**; Schöndorff, Pflüger's Arch., **54** and **63**; Gottlieb, Arch. f. exp. Path. u. Pharm., **42**; v. Jaksch, Leyden-Festschr., I, 1901.

[5] v. Schröder, Zeitschr. f. physiol. Chem., **14**; Baglioni, Centralbl. f. Physiol., **19**.

[6] Zeitschr. f. physiol. Chem., **35**, which also gives the older literature.

gram in 100 grams of blood. The blood of the portal vein contains considerably more than the blood of the arteries, being 3–4.5 times richer; this, however, is disputed by BIEDL and WINTERBERG.[1] The blood from, healthy persons contains on an average 0.90 milligram per 100 cc., according to WINTERBERG.[2] The quantity of uric acid may be 0.1 p. m. in bird's blood (v. SCHRÖDER [3]). Uric acid has not been detected with positiveness in human blood under normal conditions, while it has been found in the blood in gout, croupous pneumonia, and certain other diseased conditions. Lactic acid was first found in human blood by SOLOMON and then by GAGLIO, BERLINERBLAU, and IRISAWA. The quantity of lactic acid may vary considerably. BERLINERBLAU found 0.71 p. m. as maximum. SAITO and KATSUYAMA [4] found on an average 0.269 p. m. in hen's blood, and after carbon-monoxide poisoning the quantity increased to 1.227 p. m.

The Composition of the Blood in Different Vascular Regions and under Different Conditions.

Arterial and Venous Blood. The most striking difference between these two kinds of blood is the variation in color caused by their containing different amounts of gas and different amounts of oxyhæmoglobin and hæmoglobin. The arterial blood is light red; the venous blood is dark red, dichroic, greenish by transmitted light through thin layers. The arterial coagulates more quickly than the venous blood. The latter, on account of the transudation which takes place in the capillaries, was formerly said to be somewhat poorer in water but richer in blood-corpuscles and hæmoglobin than the arterial blood; but this is denied by modern investigators. According to KRÜGER [5] and his pupils the quantity of dry residue and hæmoglobin in blood from the carotid artery and from the jugular vein (in cats) is the same. RÖHMANN and MÜHSAM [6] could not detect any difference in the quantity of fat in arterial and venous blood.

Blood from the Portal Vein and the Hepatic Vein. In consequence of the small quantities of bile and lymph formed relatively to the large quantity of blood circulating through the liver in a given time, we can hardly expect to detect by chemical analysis a positive difference in the composition between the blood of the portal and hepatic veins. The state-

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[1] Pflüger's Arch., 88.

[2] Wien. klin. Wochenschr., 1897, and Zeitschr. f. klin. Med., **35**.

[3] Ludwig's Festschrift, 1887.

[4] Irisawa, Zeitschr. f. physiol. Chem., **17**, which also gives the older literature; Saito and Katsuyama, *ibid.*, **32**.

[5] Zeitschr. f. Biologie, **26**. This also gives the literature on the composition of the blood in different vascular regions.

[6] Pflüger's Archiv, **46**.

ments in regard to such a difference are in fact contradictory. For example, DROSDOFF found more hæmoglobin in the hepatic than in the portal vein, while OTTO found less. KRÜGER finds that the quantities of hæmoglobin, as well as of the solids, in the blood from the vessels passing to and from the liver are different, but a constant relation cannot be determined. The hepatic vein, according to DOYON and collaborators,[1] is richer in fibrinogen than the blood from the portal vein. The disputed question as to the varying quantities of sugar in the portal and hepatic veins will be discussed in a following chapter (see Chapter VIII, on the formation of sugar in the liver). After a meal rich in carbohydrates, the blood of the portal vein not only becomes richer in dextrose, but may contain also dextrin and other carbohydrates (v. MERING, OTTO[2]). The amount of urea in the blood from the hepatic vein is greater than in other blood (GRÉHANT and QUINQUAUD[3]). In regard to the quantity of ammonia, see page 318.

Blood of the Splenic Vein is decidedly richer in leucocytes than the blood from the splenic artery. The red blood-corpuscles of the blood from the splenic vein are smaller than the ordinary, less flattened, and show a greater resistance to water. The blood from the splenic vein is also claimed to be richer in water, fibrin, and protein than the ordinary venous blood. According to v. MIDDENDORFF, it is richer in hæmoglobin than arterial blood. KRÜGER[4] and his pupils found that the blood from the vena lienalis is generally richer in hæmoglobin and solids than arterial blood; still the contrary is often found. The blood from the splenic vein coagulates slowly.

The Blood from the Veins of the Glands. The blood circulates with greater rapidity through a gland during activity (secretion) than when at rest, and the outflowing venous blood has therefore during activity a lighter red color and a greater amount of oxygen. Because of the secretion the venous blood also becomes somewhat poorer in water and richer in solids.

The blood from the *Muscular Veins* shows an opposite behavior, for during activity it is darker and more venous in its properties because of the increased absorption of oxygen by the muscles and still greater production of carbon dioxide than when at rest.

Menstrual Blood, according to an old belief, has not the power of coagulating. This statement is, nevertheless, false, and the apparent uncoagulability depends in part on the retention of the blood-clot by

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[1] See footnote 2, page 245.

[2] Drosdoff, Zeitschr. f. physiol. Chem., 1; Otto, Maly's Jahresber, 17; v. Mering, Arch. f. (Anat. u.) Physiol, 1877, 214.

[3] l.c.

[4] v. Middendorff, Centralbl. f. Physiol., 2, 753; Krüger, l. c.

the womb and the vagina, so that only fluid cruor is at times eliminated, and in part on a contamination with vaginal mucus, which disturbs the coagulation. Menstrual blood, according to GAUTIER and BOURCET, contains arsenic and is also richer in iodine than other blood (see Blood-serum, page 261).

The Blood of the Two Sexes. Women's blood coagulates somewhat more quickly, has a lower specific gravity, a greater amount of water, and a smaller quantity of solids than the blood of man. The amount of blood-corpuscles and hæmoglobin is somewhat smaller in woman's blood. The amount of hæmoglobin is 146 p. m. for man's blood and 133 p. m. for woman's.

During *pregnancy* NASSE has observed a decrease in the specific gravity, with an increase in the amount of water, until the end of the eighth month. From then the specific gravity increases, and at delivery it is normal again. The amount of fibrin is somewhat increased (BECQUEREL and RODIER, NASSE). The number of blood-corpuscles seems to decrease. In regard to the amount of hæmoglobin the statements are somewhat contradictory. COHNSTEIN found the number of red corpuscles diminished in the blood of pregnant sheep as compared with non-pregnant, but the red corpuscles were larger and the quantity of hæmoglobin in the blood was greater in the first case. MÖLLENBERG [1] found in most cases an increase in the amount of hæmoglobin in pregnancy in the last months.

The Blood at Different Periods of Life. Fœtal and infant blood is richer in erythrocytes and hæmoglobin than the blood of the mother. The highest percentage of hæmoglobin in the blood has been observed by several investigators, such as COHNSTEIN and ZUNTZ, OTTO, WINTERNITZ, ABDERHALDEN, SCHWINGE, and others, immediately or very soon after birth or at least within the first few days. In man, two or three days after birth the hæmoglobin reaches a maximum (200–210 p. m.) which is greater than at any other period of life. This is the cause of the great abundance of solids in the blood of new-born infants, as observed by several investigators. The quantity of hæmoglobin and blood-corpuscles sinks gradually from this first maximum to a minimum of about 110 p. m. hæmoglobin, which minimum appears in human beings between the fourth and eighth years. The quantity of hæmoglobin then increases again until about the twentieth year, when a second maximum of 137–150 p. m. is reached. The hæmoglobin remains at this point only to about the forty-fifth year, and then gradually and slowly decreases (LEICHTENSTERN, OTTO [2]). According to earlier reports, the blood at

[1] Nasse, Maly's Jahresber., 7; Becquerel and Rodier, Traité de chim. pathol., Paris, 1854; Cohnstein, Pflüger's Arch., 34, 233; Möllenberg, Maly's Jahresber., 31, 185. See also Payer, Arch. f. Gynäk., 71.

[2] Cohnstein and Zuntz, Pflüger's Arch., 34; Winternitz, Zeitschr. f. physiol. Chem.,

old age is poorer in blood-corpuscles and protein bodies, but richer in water and salts.

The Influence of Food on the Blood. In complete starvation no decrease in the amount of solid blood-constituents is found to take place (PANUM and others). The amount of hæmoglobin is increased a little, at least in the early period (SUBBOTIN, OTTO, HERMANN and GROLL, LUCIANI and BUFALINI), and also the number of red blood-corpuscles increases (WORM MÜLLER, BUNTZEN [1]), which probably depends partly on the fact that the blood-corpuscles are not so quickly transformed as the serum and partly on a greater concentration due to loss of water. In rabbits and to a less extent in dogs, POPEL found that complete abstinence had a tendency to increase the specific gravity of the blood. The amount of fat in the blood may be somewhat increased in starvation because the fat is taken up from the fat deposits and carried to the various organs by the blood (N. SCHULZ, DADDI [2]).

After a rich meal, or after secretion of digestive juices or absorption of nutritive liquids, the relative number of blood-corpuscles may be increased or diminished (BUNTZEN, LEICHTENSTERN). The number of white blood-corpuscles may be considerably increased after a diet rich in proteins. After a diet rich in fat the plasma becomes, even after a short time, more or less milky-white, like an emulsion. The composition of the food acts essentially on the amount of hæmoglobin in the blood. The blood of herbivora is generally poorer in hæmoglobin than that of carnivora, and SUBBOTIN has observed in dogs after a partial feeding with food rich in carbohydrates that the amount of hæmoglobin sank from the physiological average of 137.5 p. m. to 103.2–93.7 p. m. TSUBOI [3] has also shown in experiments on rabbits and dogs that with an insufficient diet of bread and potatoes, where the body gave up protein and contained relatively considerable carbohydrate, the amount of hæmoglobin decreased and the blood became richer in water. According to LEICHTENSTERN, a gradual increase in the amount of hæmoglobin is found to take place in the blood of human beings on enriching the food, and according to the same investigator the blood of lean persons is generally some-

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22; Leichtenstern, Untersuch. über den Hämoglobingehalt des Blutes, etc., Leipzig, 1878; Otto, Maly's Jahresber., 15 and 17; Abderhalden, Zeitschr. f. physiol. Chem., 34; Schwinge, Pflüger's Arch., 73 (literature). See also Fehrsen, Journ. of Physiol., 30.

[1] Panum, Virchow's Arch., 29; Subbotin, Zeitschr. f. Biologie, 7; Otto, l. c.; Worm Müller, Transfusion und Plethora, Christiania, 1875; Buntzen, see Maly's Jahresber., 9; Hermann and Groll, Pflüger's Arch., 43; Luciani and Bufalini, Maly's Jahresber., 12.

[2] Popel, Arch. des scienc. biol. de St. Pétersbourg, 4, 354; Schulz, Pflüger's Arch., 65; Daddi, Maly's Jahresber., 30.

[3] Subbotin, l. c.; Tsuboi, Zeitschr. f. Biologie, 44.

what richer in hæmoglobin than blood from fat ones of the same age. The addition of iron salts to the food greatly influences the number of blood-corpuscles and especially the amount of hæmoglobin they contain. The action of the iron salts is obscure.[1] There does not seem to be any doubt that the iron contained in the food in the form of organic compounds is active, but also iron salts and therapeutic iron. According to BUNGE and his pupils the iron preparations act indirectly only. They may combine with the sulphuretted hydrogen of the intestinal canal and thereby prevent the iron associated in the food as assimilable protein compounds from being eliminated as iron sulphide (BUNGE), a view which is now generally discarded.

An *increase in the number of red corpuscles*, a true "*plethora polycythæmia*," takes place after transfusion of blood of the same species of animal. According to the observations of PANUM and WORM MÜLLER,[2] the blood-liquid is quickly eliminated and transformed in this case—the water being eliminated principally by the kidneys and the protein burned into urea, etc.—while the blood-corpuscles are preserved longer and cause a "*polycythæmia*." A relative increase in the number of red corpuscles is found after abundant transudation from the blood, as in cholera and heart-failure with considerable congestion. An increase in the number of red blood-corpuscles has also been observed under the influence of diminished pressure or in high altitudes. VIAULT first called attention to the fact that the number of red corpuscles was very great in the blood of man and animals living in high regions. According to him the llama has about 16 million blood-corpuscles per cubic millimeter. By observations on himself and others, as well as on animals, VIAULT found the first effect of sojourning in high altitudes was a very considerable increase in the number of red corpuscles, in his own case 5–8 millions. A similar increase of the red blood-corpuscles, as also an increase in the quantity of hæmoglobin under the influence of diminished pressure, has been observed by many other investigators in human beings as well as in animals. Investigators are not united as to how this increase is brought about. The increase in the blood-corpuscles is not absolute, but is only relative, and it is considered by several observers that there is neither a new formation nor a diminished destruction of the blood-corpuscles. A relative increase may be brought about in different ways. For example, another division of the blood-corpuscles in the vascular system has been supposed, whereby the blood-corpuscles accumulate

[1] See Bunge, Zeitschr. f. physiol. Chem., **9**; Häusermann, *ibid.*, **23**, where the works of Woltering, Gaule, Hall, Hochhaus, and Quincke are cited (the same work contains a table of the quantity of iron in various foods); Kunkel, Pflüger's Arch., **61**; Macallum, Journal of Physiol., **16**; Abderhalden, Zeitschr. f. Biologie, **39**.

[2] Panum, Virchow's Arch., **29**; Worm Müller, l. c.

in the capillaries, from which region the blood has been examined most often (ZUNTZ). It is also claimed that a concentration of the blood takes place by increased evaporation (GRAWITZ), and finally an increase in the blood-corpuscles has also been explained by assuming a contraction of the vascular system with the pressing out of plasma (BUNGE, ABDERHALDEN [1]). In connection with these experiments, it must be remarked that several trustworthy observations show that under the influence of diminished blood-pressure an actual increase in the red blood-corpuscles takes place, and ZUNTZ [2] and his co-workers have also shown that the activity in the red bone-marrow is increased.

A *decrease in the number of red corpuscles* occurs in anæmia from different causes. Every excessive hemorrhage causes an acute anæmia, or, more correctly, oligæmia. Even during the hemorrhage, the remaining blood becomes by diminished secretion and excretion, as also by an abundant absorption of parenchymous fluid, richer in water, somewhat poorer in proteins, and strikingly poorer in red blood-corpuscles. The oligæmia soon passes into a hydræmia. The amount of protein then gradually increases again; but the reformation of the red blood-corpuscles is slower, and after the hydræmia follows also an oligocythæmia. After a little time the number of blood-corpuscles rises to normal. INAGAKI [3] has made thorough investigations on the changes which the number, volume and hæmoglobin content of the erythrocytes undergo after drawing blood as well as during regeneration. It is impossible here to enter more in detail as to the results, but simply to state that they substantiate the previously known observation that during regeneration irregularities may occur in the relation between the quantity of hæmoglobin and the number of erythrocytes. This he explains by the fact, as claimed by BOHR (see page 271), that there exist different hæmoglobins containing different quantities of iron. A considerable decrease in the number of red corpuscles also occurs in chronic anæmia and chlorosis; still in such cases an essential decrease in the amount of hæmoglobin occurs without an essential decrease in the number of blood-corpuscles. The decrease in the amount of hæmoglobin is more characteristic of chlorosis than a decrease in the number of red corpuscles. The opinions on the changes in the blood in anæmia and chlorosis differ very considerably.[4]

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[1] The literature on this subject may be found in Abderhalden, Zeitschr. f. Biologie, **43**; van Voornveld, Pflüger's Arch., **92**.

[2] Höhenklima und Bergwanderungen, by N. Zuntz, A. Loewy, Franz Müller, and W. Caspari, Berlin, 1906.

[3] Zeitschr. f. Biol., **49**.

[4] Complete analyses of chlorotic blood may be found in Erben, Zeitschr. f. klin. Med., **47**.

A very considerable decrease in the number of red corpuscles (300,000–400,000 in 1 c.mm.) and diminution in the amount of hæmoglobin ($\frac{1}{3}$–$\frac{1}{10}$) occurs in pernicious anæmia (HAYEM, LAACHE, and others). On the contrary, the individual red corpuscles are larger and richer in hæmoglobin than they ordinarily are, and the number stands in an inverse relation to the amount of hæmoglobin (HAYEM). Besides this the red corpuscles often, but not always, show in pernicious anæmia remarkable and extraordinary irregularities of form and size, which QUINCKE [1] has termed *poikilocytosis*.

The *number of leucocytes* may, as stated above, be increased under physiological conditions as well as after a meal rich in protein (physiological leucocytosis). Under pathological conditions a high leucocytosis may occur, and this is especially found in leucæmia, which is characterized by a very great abundance of leucocytes in the blood. The number of leucocytes is markedly increased in this disease, and indeed, not only absolutely, but also in relation to the number of red blood-corpuscles, which are increased to a considerable extent in leucæmia. Leucæmic blood has a lower specific gravity than the ordinary blood (1035–1040), and a paler color, as if it were mixed with pus. The reaction is alkaline, but after death it is frequently acid, probably due to a decomposition of lecithin, which is often considerably increased in leucæmia. Volatile fatty acids, lactic acid, glycero-phosphoric acid, large amounts of purine bases, and so-called CHARCOT'S crystals (see Semen, Chapter XIII) have also been found in leucæmic blood. The peptone (proteose) which is found in the leucæmic blood after death, and which does not exist in the fresh blood, is, according to ERBEN,[2] a digestive product which is produced by a tryptic enzyme which originates from the leucocytes as well as by traces of a peptic enzyme. A chemical analysis of leucæmic blood has recently been made by ERBEN.[3]

A great number of investigations have been made on the chemical composition of blood in disease. But as we have only a few analyses of the blood of healthy individuals, and as the possible variations under physiological conditions are little known, it is difficult to draw any positive conclusions from the analyses of pathological blood. Unfortunately, on account of the large number of contradictory deductions concerning the composition of the blood of diseased human beings, it is impossible

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[1] Laache, Die Anämie (Christiania, 1883), which also contains the literature; Quincke, Deutsch. Arch. f. klin. Med., **20** and **25**. A complete chemical analysis of the blood has been made by Erben, Zeitschr. f. klin. Med., **40**.

[2] Erben, Zeitschr. f. Heilkunde, **24**, and Hofmeister's Beiträge, **5**. See also Schumm, *ibid.*, **4** and **5**. See also footnote 6, page 295.

[3] Zeitschr. f. klin. Med., **66** (1908).

to give a brief summary of the results, still the changes in the blood in disease must be of the greatest importance.

The *quantity of blood* is indeed somewhat variable in different species of animals and in different conditions of the body; in general we consider the entire quantity of blood in adults as about $\frac{1}{13}$–$\frac{1}{14}$ of the weight of the body, and in new-born infants about $\frac{1}{19}$. HALDANE and LORRAIN SMITH,[1] who have determined the quantity of blood by a new method, find in fourteen persons that it varies between $\frac{1}{16}$ and $\frac{1}{30}$ of the weight of the body. According to the same method OERUM[2] has determined the quantity of blood in men as about $\frac{1}{19}$ and in women $\frac{1}{22}$ of the weight of the body. Fat individuals are relatively poorer in blood than lean ones. During inanition the quantity of blood decreases less quickly than the weight of the body (PANUM[3]), and it may therefore be also proportionally greater in starving individuals than in well-fed ones.

By careful bleeding the quantity of blood may be considerably diminished without any dangerous symptoms. A loss of blood amounting to one-fourth of the normal quantity has as a sequence no lasting sinking of the blood-pressure in the arteries, because the smaller arteries accommodate themselves to the small quantities of blood by contracting (WORM MÜLLER[4]). A loss of blood amounting to one-third of the quantity reduces the blood-pressure considerably, and a loss of one-half of the blood in adults is dangerous to life. The more rapid the bleeding the more dangerous it is. New-born infants are very sensitive to loss of blood, and likewise fat, old, and weak persons cannot stand much loss of blood. Women can stand loss of blood better than men.

The quantity of blood may be considerably increased by the injection of blood from the same species of animal (PANUM, LANDOIS, WORM MÜLLER, PONFICK). According to WORM MÜLLER the normal quantity of blood may indeed be increased as much as 83 per cent without producing any abnormal conditions or lasting high blood-pressure. An increase of 150 per cent in the quantity of blood may, with a considerable variation in the blood-pressure, be directly dangerous to life (WORM MÜLLER). If the quantity of blood of an animal is increased by transfusion with blood of the same kind of animal, an abundant formation of lymph takes place. The water in excess is eliminated by the urine; and as the protein of the blood-serum is quickly decomposed, while the red blood-corpuscles are destroyed much more slowly (TSCHIRJEW, FORSTER, PANUM, WORM MÜLLER[5]), a polycythæmia is gradually produced.

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[1] Journ. of Physiol., **25**.

[2] Deutsch. Arch. f. klin. Med., **93** (1908).

[3] Virchow's Arch., **29**.

[4] Transfusion und Plethora, Christiania, 1875.

[5] Panum, Nord. med. Ark., **7**; Virchow's Arch., **63**; Landois, Centralbl. f. d. med.

The quantity of blood in the different organs depends essentially on their activity. During work the exchange of material in an organ is more pronounced than during rest, and the increased metabolism is connected with a more abundant flow of blood. Although the total quantity of blood in the body remains constant, the distribution of the blood in the various organs may be different at different times. As a rule the quantity of blood in an organ is an approximate measure of the more or less active metabolism going on in it, and from this point of view the distribution of the blood in the different organs is of interest. According to RANKE,[1] to whom we are especially indebted for our knowledge of the relation of the activity of the organs to the quantity of blood contained therein, of the total quantity of blood (in the rabbit) about one-fourth comes to the muscles in rest, one-fourth to the heart and the large blood-vessels, one-fourth to the liver, and one-fourth to the other organs.

Wissensch., 1875, and Die Transfusion des Blutes, Leipzig, 1875; Worm Müller, Transfusion und Plethora; Ponfick, Virchow's Arch., **62**; Tschirjew, Arbeiten aus der physiol. Anstalt zu Leipzig, 1874, 292; Forster, Zeitschr. f. Biologie, **11**; Panum, Virchow's Arch., **29**.

[1] Die Blutvertheilung und der Thätigkeitswechsel des Organe, Leipzig, 1871.

CHAPTER VII.

CHYLE, LYMPH, TRANSUDATES AND EXUDATES.

I. CHYLE AND LYMPH.

THE lymph is at least in part the mediator in the exchange of constituents between the blood and the tissues. The bodies necessary for the nutrition of the tissues pass from the blood into the lymph, and the tissues deliver water, salts, and products of metabolism to the lymph. The lymph, therefore, originates partly from the blood and partly from the tissues. From a purely theoretical standpoint one can, according to HEIDENHAIN, differentiate between blood-lymph and tissue-lymph according to origin. It is impossible at the present time to completely separate that which comes from the one or the other source.

Chemically the lymph is the same as plasma and contains, at least to a great extent, the same bodies. The observation of ASHER and BARBÈRA,[1] that the lymph contains poisonous metabolic products, does not contradict such an assumption, as no doubt these products are transferred to the blood with the lymph. Although the blood does not show the same poisonous action as the lymph, still this can be explained by the great dilution these bodies undergo in the blood, and the difference between blood-plasma and lymph is no doubt of a quantitative nature. This difference consists chiefly in that the lymph is poorer in proteins. No essential chemical difference has been found between the lymph and the chyle of starving animals. After fatty food the chyle differs from the lymph in its wealth of minutely divided fat-globules, which gives it a milky appearance; hence the old name "lacteal fluid."

Chyle and lymph, like the plasma, contain *seralbumin*, *serglobulins*, *fibrinogen*, and *fibrin ferment*. The two last-mentioned bodies occur only in very small amounts; therefore the chyle and lymph coagulate slowly (but spontaneously) and yield but little fibrin. Like other liquids poor in fibrin ferment, chyle and lymph do not at once coagulate completely, but repeated coagulations take place.

The extractive bodies seem to be the same as in plasma. *Sugar* (or at least a reducing substance) is found in about the same quantity as in

[1] Zeitschr. f. Biologie, **36**.

the blood-serum, but in larger quantities than in the blood; this depends on the fact that the blood-corpuscles contain no sugar. The *glycogen* detected by DASTRE[1] in the lymph occurs only in the leucocytes. According to RÖHMANN and BIAL, lymph contains a diastatic enzyme similar to that in blood-plasma, and LÉPINE[2] found that the chyle of a dog during digestion has great glycolytic activity. The amount of *urea* has been determined by WURTZ[3] as 0.12–0.28 p. m. The *mineral bodies* appear to be the same as in plasma.

As form-elements, *leucocytes* and *red blood-corpuscles* are common to both chyle and lymph. Chyle in fasting animals has the appearance of lymph. After fatty food it is, on the contrary, milky, due partly to small fat-globules, as in milk, and partly, indeed, mostly to finely divided fat. The nature of the *fat* occurring in chyle depends upon the kind of fat in the food. By far the greater part consists of neutral fat, and even after feeding with large quantities of free fatty acids, MUNK[4] found that the chyle contained chiefly neutral fat with only small amounts of fatty acids or soaps.

The *gases* of the chyle have not been studied, and it seems that the gases of an entirely normal human lymph have not thus far been investigated. The gases from dog-lymph contain, according to HAMMARSTEN, only traces of oxygen, and consist of 37.4–53.1 per cent CO_2 and 1.6 per cent N, calculated at 0° C., and 760 mm. mercury. The chief mass of the carbon dioxide of the lymph seems to be in firm chemical combination. Comparative analyses of blood and lymph have shown that the lymph contains more carbon dioxide than arterial, but less than venous, blood. The tension of the carbon dioxide of lymph is, according to PFLÜGER and STRASSBURG,[5] smaller than in venous, but greater than in arterial, blood.

The *quantitative composition of the chyle* must evidently be very variable.[6] The analyses thus far made refer only to that mixture of chyle and lymph which is obtained from the thoracic duct. The specific gravity varies between 1.007 and 1.043. As an example of the composition of human chyle two analyses will be given. The first is by OWEN-REES, of the chyle of an executed person, and the second by

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[1] Compt. rend. de soc. biol., **47**, and Compt. rend., **120**; Arch. de Physiol. (5), **7**.

[2] Röhmann and Bial, Pflüger's Arch., **52**, **53**, and **55**; Lépine, Compt. rend., **110**.

[3] Compt. rend., **49**.

[4] Virchow's Arch., **80** and **123**. In regard to the analysis of the fat of chyle, see Erben, Zeitschr. f. physiol. Chem., **30**.

[5] Hammarsten, Die Gase der Hundelymphe, Arbeiten aus d. physiol. Anstalt zu Leipzig, 1871; Strassburg, Pflüger's Archiv, **6**.

[6] See also Panzer, Zeitschr. f. physiol. Chem., **30**.

HOPPE-SEYLER,[1] of the chyle in a case of rupture of the thoracic duct. In the latter case the fibrin had previously separated. The results are in parts per 1000.

	No. 1.	No. 2.
Water	904.8	940.72 water
Solids	95.2	59.28 solids
Fibrin	Traces	.....
Albumin	70.8	36.67 albumin
Fat	9.2	7.23 fat
Remaining organic bodies	10.8	2.35 soaps 0.83 lecithin 1.32 cholesterin 3.63 alcohol extractives 0.58 water extractives
Salts	4.4	6.80 soluble salts 0.35 insoluble salts

The quantity of fat is very variable and may be considerably increased by partaking of food rich in fats. I. MUNK and A. ROSENSTEIN[2] have investigated the lymph or chyle obtained from a lymph fistula at the end of the upper third of the leg of a girl eighteen years old and weighing 60 kg., and the highest quantity of fat in the chylous lymph was 47 p. m. after partaking of fat. In the starvation lymph from the same patient they found only 0.6–2.6 p. m. fat. The quantity of soaps was always small, and on partaking of 41 grams of fat the quantity of soaps was only about $\frac{1}{20}$ of the neutral fats. SCHUMM[3] found in the creamy contents of a chylous cyst of the mesentery 357.8 p. m. fat and comparatively large amounts of calcium soaps.

A great many analyses of chyle from animals have been made, and they chiefly show the fact that the chyle is a liquid with a very changeable composition which stands closely related to blood-plasma, but with the chief difference that it contains more fat and less solids. The reader is referred to special works for these analyses, as, for example, to v. GORUP-BESANEZ'S "Lehrbuch der physiologischen Chemie," 4th edition.

The *composition of the lymph* is also very changeable, and its specific gravity shows about the same variation as the chyle. In the following analyses, 1 and 2, made by GUBLER and QUEVENNE, are the results obtained from lymph from the upper part of the thigh of a woman aged thirty-nine; and 3, made by v. SCHERER, is an analysis of lymph from the sac-like dilated lymphatic vessels of the spermatic cord. No. 4 was made

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[1] Owen-Rees, cited from Hoppe-Seyler's Physiol. Chem., 595; Hoppe-Seyler, *ibid.*, 597. See also Carlier, Brit. Med. Journ., 1902, 175, and T. Sollmann, Amer. Journ. of Physiol., **17**.

[2] Virchow's Arch., **123**.

[3] Zeitschr. f. physiol. Chem., **49**.

by C. SCHMIDT,[1] the data being obtained from lymph from the neck of a colt. The results are expressed in parts per 1000.

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0.4	2.2
Albumin	42.7	42.8	34.7	....
Fat, cholesterin, lecithin	3.8	9.2	....	35.0
Extractive bodies	5.7	4.4	....	....
Salts	7.3	8.2	7.2	7.5

The salts found by C. SCHMIDT in the lymph of the horse have the following composition, calculated in parts per 1000 parts of the lymph:

Sodium chloride	5.67
Soda	1.27
Potash	0.16
Sulphuric acid	0.09
Phosphoric acid united with alkalies	0.02
Earthy phosphates	0.26

In the cases investigated by MUNK and ROSENSTEIN the quantity of solids in the fasting condition varied between 35.7 and 57.2 p. m. This variation was essentially dependent upon the extent of secretion, so that the low amount coincides with a more active secretion, and the reverse in the other case. The chief portion of the solids consisted of proteins, and the relation between globulin and albumin was as 1:2.4 to 4. The mineral bodies in 1000 parts lymph (chylous) were: NaCl 5.83; Na_2CO_3 2.17; K_2HPO_4 0.28; $Ca_3(PO_4)_2$ 0.28; $Mg_3(PO_4)_2$ 0.09; and Fe (PO_4) 0.025. CARLSON, GREEN and LUCKHARDT[2] have recently made comparative estimations of NaCl in blood-serum and lymph of the same individual (horse and dog) and find that the lymph is regularly richer in chlorides, a condition which, according to them, is difficult to reconcile with the view of the filtration and transudation processes in the formation of lymph.

Under special conditions the lymph may be so rich in finely divided fat that it appears like chyle. Such lymph has been investigated by HENSEN in a case of lymph fistula in a ten-year-old boy, and by LANG[3] in a case of lymph fistula in the upper part of the left thigh of a girl of seventeen. The lymph investigated by HENSEN varied in the quantity of fat, as an average of nineteen analyses, between 2.8 and 36.9 p. m.; while that investigated by LANG contained 24.85 p. m. of fat.

The quantity of lymph secreted must naturally change considerably under various conditions, and there are no means of measuring it. The

[1] Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 591; v. Scherer, *ibid.*, 591; C. Schmidt, *ibid.*, 592.

[2] Amer. Journ. of Physiol., **22** (1908).

[3] Hensen, Pflüger's Arch., **10**; Lang, see Maly's Jahresber., **4**.

size of the flow of lymph is, as HEIDENHAIN suggests, no measure of the abundance of supply of nutritive material to the organs, and the lymph-tubes act according to him as "drain-tubes," removing the excess of fluid from the lymph-fissures as soon as the pressure therein rises to a certain height. Attempts have been made to determine the quantity of lymph flowing in 24 hours in the thoracic duct of animals. According to HEIDENHAIN the quantity averages 640 cc. for a dog weighing 10 kilos.

Determinations of the quantity of lymph in man have also been attempted. NOËL-PATON [1] obtained 1 cc. of lymph per minute from the severed thoracic duct of a patient weighing 60 kilos. The quantity in the 24 hours cannot be calculated from this amount. In the case of MUNK and ROSENSTEIN, 1134–1372 grams of chyle were collected within 12–13 hours after partaking of food. In the fasting condition or after starving for 18 hours they found 50 to 70 grams per hour, sometimes 120 grams and above, especially in the first few hours after powerful muscular exercise.

Several circumstances have a marked influence on the extent of lymph secretion. During starvation less lymph is secreted than after partaking of food. NASSE [2] has observed that the formation of lymph in dogs is increased 36 per cent more after feeding with meat than after feeding with potatoes, and about 54 per cent more than after 24 hours' deprivation of food. In this connection mention must be made of the important observations of ASHER and BARBÈRA [3] that with pure protein diet the lymph current is increased in the thoracic cavity, and also that the increase in the lymph secretion runs parallel with the elimination of nitrogen in the urine, i.e., with the absorption of the protein from the digestive tract.

An increase in the total quantity of blood, as by transfusion of blood, also especially on preventing the flow of blood by means of ligatures, causes an increase in the quantity of lymph. According to HEIDENHAIN, on the contrary, a very considerable change in the pressure in the aorta causes only a little change in the abundance of the lymph-flow. The quantity of lymph may be raised by powerfully active and passive movements of the limbs (LESSER). Under the influence of curare, an increase of the lymph secretion is observed (PASCHUTIN, LESSER [4]), and the quantity of solids in the lymph is also increased.

The bodies inciting lymph-flow, the so-called *lymphagogues*, are of

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[1] Journ. of Physiol., **11**.

[2] Cited from Hoppe-Seyler, Physiol. Chem., 593.

[3] The works of Asher and collaborators, Barbèra, Gies, and Busch, upon lymph formation may be found in Zeitschr. f. Biologie, **36**, **37**, **40**.

[4] Lesser, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang **6**; Paschutin, *ibid.*, **7**.

especially great interest, and they may, according to HEIDENHAIN,[1] be divided into two different chief groups. The lymphagogues of the first series—extracts of crab-muscles, blood-leech, anodons, liver and intestine of dogs, as well as peptone and egg albumin, strawberry extracts, metabolic products of bacteria and others—cause a greatly increased secretion of lymph without raising the blood-pressure, and in this way the blood-plasma becomes poorer in proteins and the lymph richer than before. For the formation of this lymph, which HEIDENHAIN designates blood-lymph, we must admit with him that a special secretory activity of the capillary-wall endothelium exists. The lymphagogues of the second series, such as sugar, urea, sodium chloride, and other salts, also cause an abundant lymph formation. The blood, as well as the lymph, thereby becomes richer in water. This increased amount of water depends, according to HEIDENHAIN, upon an increased delivery of water by the tissue-elements, and this lymph is chiefly tissue-lymph, in his opinion. Diffusion is no doubt of great importance in the formation of this lymph, but the secretory activity of the endothelium is also of importance, at least for certain bodies, such as sugar.

In the past, the formation of lymph was explained in a purely physical way by the united action of filtration from the blood and the osmosis between the blood and tissue-fluid. Later HEIDENHAIN and also HAMBURGER ascribed a special activity to the capillary endothelium, assuming that they take part in the formation of lymph in a secretory manner.

Another view, which besides the physical processes is also of especial physiological moment in the explanation of lymph formation, was suggested by ASHER and his collaborators (BARBÈRA, GIES, and BUSCH). According to them the lymph is a product of the work of the organs; its amount is dependent upon an increased or diminished activity of the organs, and the lymph is therefore a measure of the work in these. The close relation between lymph formation and the work of organs has also been shown for several of them, especially for the liver. STARLING has shown that after the introduction of lymphagogues of the first series, chiefly liver lymph is secreted, which he claims is a proof against HEIDENHAIN'S view, and he explains the increased permeability of the vessel wall by the fact that these bodies have an irritating, poisonous action. On the contrary, ASHER explains this increased lymph flow by the statement that the substance in question—as well as those influences which incite the activity of the liver—produces an increased formation of lymph in these organs. This view is supported by experiments upon the action

[1] Heidenhain, Pflüger's Arch., **49**; Hamburger, Zeitschr. f. Biologie, **27** and **30**. See especially Ziegler's Beitr. zur Path. u. zur allg. Pathol., **14**, 443; also Arch. f. (Anat. u.) Physiol., 1895 and 1896.

of lymphagogues on blood coagulation and liver activity (DELEZENNE and others), for, according to GLEY, these bodies have at the same time a lymphagogue action and an action upon the secretion of the glands. We have no direct evidence of the action of the lymphagogues of the first series upon the organs, but we know from KUSMINE'S work that peptone, leech extract, and the extractives of the crab-muscles act directly upon the liver-cells and bring about morphological changes. The connection between organ activity and lymph formation has also been shown upon muscles and glands by others besides the above-mentioned investigators (HAMBURGER, BAINBRIDGE [1]).

The extent of organ work certainly essentially influences the quantity and properties of the lymph. Still from this we cannot draw any positive conclusions as to whether the lymph formation is brought about by physico-chemical processes alone or whether in this process a specific, not closely definable secretory force is at work at the same time. In regard to this much-disputed question attention must be called in the first place to the fact that the important works of HEIDENHAIN, HAMBURGER, LAZARUS-BARLOW, and others, as well as the investigations of ASHER and GIES and of MENDEL and HOOKER [2] upon the lengthy post-mortem lymph-flow, have shown that the older filtration hypothesis is untenable. That the part played by filtration as compared with that of the osmotic force is only very trivial has been conclusively shown by the adherents of the physico-chemical theory of lymph formation.

Several investigators (KORÁNYI, STARLING, ROTH, ASHER, and others) have clearly shown that the work in the glands and tissue-cells must cause a difference in the osmotic pressure upon the two sides of the capillary wall. That this is so follows from several circumstances, and especially from the fact that, in disassimilation in the cells, bodies of high molecular weight are split into a number of smaller molecules, which latter, either directly, if they leave the cells and pass into the tissue-fluid, or indirectly, when they remain in the cells, produce an increase in the osmotic tension within the cells, and in this way cause a taking up of water from the fluid, and must therefore increase the osmotic pressure of the tissue-fluids. As the cells can by synthesis build up highly complex constituents from simple molecules, and as the chief products of catabolism are carbon dioxide and water, it is difficult to explain these intricate conditions. Still, irrespective of whatever view, a change in one or the other direction in the osmotic pressure upon both sides of the capillary wall must be produced thereby. Whether this and other physico-chemical processes

[1] In regard to the works cited, as well as the literature upon lymph formation, see Ellinger, "Die Bildung der Lymphe," Ergebnisse der Physiol., I, Abt. 1, 1902, and Asher, Biochem. Centralbl., 4.

[2] Amer. Journ. of Physiol., 7. See also footnote 1.

are alone sufficient to explain the lymph formation (COHNSTEIN, ELLINGER) remains an open and disputed question.[1]

II. TRANSUDATES AND EXUDATES.

The serous membranes are normally kept moistened by liquids whose quantity is sufficient only in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under diseased conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues, or under the epidermis; and in this way pathological transudates are formed. Such true transudates, which are similar to lymph, are generally poor in form-elements and leucocytes, and yield only very little or almost no fibrin, while the inflammatory transudates, the so-called exudates, are generally rich in leucocytes and yield proportionally more fibrin. As a rule, the richer a transudate is in leucocytes the closer it stands to pus, while a diminished quantity of leucocytes renders it more nearly like a real transudate or lymph.

It is ordinarily accepted that filtration is of the greatest importance in the formation of transudates and exudates. The facts coincide with this view that all these fluids contain the salts and extractive bodies occurring in the blood-plasma in about the same quantity as the blood-plasma, while the amount of proteins is habitually smaller. While the different fluids belonging to this group have about the same quantities of salts and extractive bodies, they differ from one another chiefly in containing differing quantities of protein and form-elements, as well as varying quantities of transformation and decomposition products of these latter—changed blood-coloring matters, cholesterin, etc. The correspondence in the amount of salts and extractive bodies present in the blood and in transudates supplies just as little proof for a filtration as it does for the formation of lymph; but still it cannot be doubted for other reasons that filtration is often of great importance in the formation of a transudate. To what extent filtration is active in the perfectly normal vascular wall cannot be answered.

The altered permeability of the capillary walls in disease is a second important factor in the formation of transudates. The circumstance that the greatest quantity of protein occurs in transudates in inflammatory processes, to which is also due the abundant quantity of form-elements in such transudates, has been explained by this hypothesis. The greater quantity of protein in the transudates in formative irritation is in great part explained by the large amount of destroyed form-elements. The

[1] On this question see Ellinger, "Bie Bildung der Lymphe," Ergebnisse der Physiologie, I, Abt. 1, 355, and Asher, Biochem. Centralbl., 4, pp. 1 and 45.

interesting observation made by PAIJKULL,[1] that in those cases in which an inflammatory irritation has taken place the fluid contains nucleoalbumin (or nucleoprotein?), while this substance does not occur in transudates in the absence of inflammatory processes, can be explained by the presence of form-elements. Still, such a phosphorized protein substance does not occur in all inflammatory exudates.

As the secretory importance of the capillary endothelium has been made probable by the investigations of HEIDENHAIN, it is *a priori* to be expected that an abnormally increased secretory activity of the endothelium is a cause of transudates. Those observations which substantiate such an assumption can also be explained just as well by assuming a changed permeability of the capillary walls.

The varying quantities of protein observed by C. SCHMIDT[2] in the tissue-fluids in different vascular regions can perhaps be explained by the different condition of the capillary endothelium. For example, the amount of protein in the PERICARDIAL, PLEURAL, and PERITONEAL FLUIDS is considerably greater than in those fluids which are found in the SUBARACHNOIDAL SPACE, in the SUBCUTANEOUS TISSUES, or in the AQUEOUS HUMOR, which are poor in protein. The condition of the blood also greatly affects the transudates, for in hydræmia the amount of protein in the transudate is very small. With the increase in the age of a transudate, of a hydrocele fluid for instance, the quantity of protein is increased, probably by resorption of water, and indeed exceptional cases may occur in which the amount of protein, witnout any previous hemorrhage, is even greater than in the blood-serum.

The proteins of transudates are chiefly seralbumin, serglobulin, and a little fibrinogen. Proteoses and peptones do not occur, excepting perhaps in the cerebrospinal fluid, and in these cases where an autolysis has taken place in the liquid.[3] The non-inflammatory transudates do not as a rule undergo spontaneous coagulation only very slowly, or not at all. On the addition of blood or blood-serum they coagulate. Inflammatory exudates coagulate spontaneously, and PAIJKULL has shown that these often contain nucleoprotein (or nucleoalbumin). In inflammatory exudates a protein substance has been habitually observed which is precipitated by acetic acid, but which does not occur in transudates, or only in very small quantities. This substance, which has been observed and studied by MORITZ, STAEHELIN, UMBER, and RIVALTA, is claimed by the first three observers to be free from phosphorus, while RIVALTA

[1] See Maly's Jahresber., **22**.

[2] Cited from Hoppe-Seyler, Physiol. Chem., 607.

[3] Umber, Münch. med. Wochenschr., 1902, and Berlin. klin. Wochenschr., 1903. In regard to the autolysis in transudates, see also Galdi, Biochem. Centralbl., **3**; Eppinger, Zeitschr. f. Heilkunde, **35** and Zak, Wien. klin. Wochenschr., 1905.

considers it to be a phosphorized pseudoglobulin. UMBER calls it *serosamucin*, although it yields only very little reducing carbohydrate. According to JOACHIM [1] it is only a part of the globulin, a view which cannot be correct for all cases. v. HOLST [2] has so far substantiated UMBER'S observation in that he has isolated a mucin substance from an ascitic fluid in carcinoma of the stomach and the peritoneum, which seemed to be identical with UMBER'S serosamucin, as well as with the synovial mucin. There does not seem to be any doubt that in transudates and exudates different protein substances may occur under different circumstances, although the globulins form besides seralbumin ordinarily the chief mass of the protein bodies. Mucoid substances, which were first observed by HAMMARSTEN in certain cases of ascites without complications with ovarial tumors, and which are cleavage products of a more complicated substance, seem according to PAIJKULL [3] to be regular constituents of transudates and are closely related to the above-mentioned serosamucin.

There are numerous investigations on the relation between globulin and seralbumin, and JOACHIM has recently determined the relation between euglobulin and the total globulin. No conclusive results can be drawn from these determinations. The relation between globulin and seralbumin varies very much in different cases, but, as HOFFMANN and PIGEAND [4] have shown, the variation is in each case the same as in the blood-serum of the individual.

The specific gravity runs nearly parallel with the quantity of protein. The varying specific gravity has been suggested as a means of differentiation between transudates and exudates by REUSS,[5] as the first often show a specific gravity below 1015–1010, while the others have a specific gravity of 1018 or above. This rule holds good in many, but not in all cases.

The *gases* of the transudates consist of carbon dioxide besides small amounts of nitrogen and traces of oxygen. The tension of the carbon dioxide is greater in the transudates than in the blood. When mixed with pus, the amount of carbon dioxide is decreased.

The *extractives* are, as above stated, the same as in the blood-plasma. *Urea* seems to occur in very variable amounts. *Sugar* also occurs in transudates, but it is not known to what extent the reducing power is

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[1] Paijkull, l. c.; Moritz, Münch. med. Wochenschr., 1903; Staehelin, *ibid.*, 1902; Umber, Zeitschr. f. klin. Med., **48**; Rivalta, Biochem. Centralbl., **2** and **5**; Joachim, Pflüger's Arch., **93**.

[2] Zeitschr. f. physiol. Chem., **43**.

[3] Hammarsten, *ibid.*, **15**; Paijkull, l. c.

[4] Joachim, l. c.; Hoffmann, Arch. f. exp. Path. u. Pharm., **16**; Pigeand, see Maly's Jahresber., **16**.

[5] Reuss, Deutsch. Arch. f. klin. Med., **28**. See also Otto, Zeitschr. f. Heilkunde, **17**.

due to other bodies, as in blood-serum. A reducing, non-fermentable substance has been found by PICKARDT in transudates. The sugar is generally dextrose, but levulose seems to have been found[1] in several cases. *Sarcolactic acid* has been found by C. KÜLZ[2] in the pericardial fluid from oxen. *Succinic acid* has been found in a few cases in hydrocele fluids, while in other cases it is entirely absent. *Leucine* and *tyrosine* have been found in transudates from diseased livers and pus-like transudates which have undergone decomposition, and after autolysis. Among other extractives found in transudates must be mentioned *allantoin* (MOSCATELLI[3]), *uric acid, purine bases, creatine, inosite,* and *pyrocatechin* (?).

The division of the nitrogenous substances in human transudates and exudates has so far been little studied. OTORI[4] found that no essential difference exists between serous exudates and transudates in regard to the quantity of urea and amino-acids. The amount of total nitrogen and proteins runs parallel with the specific gravity, and the same is generally true for the absolute values for amino-acid nitrogen and purine nitrogen. The amino-acid nitrogen and the urea nitrogen in pus is greater as the specific gravity rises, but not in serous exudates and transudates.

The investigations upon the molecular concentration have shown that no essential and constant difference exists between exudates and transudates. The osmotic concentration and the concentration of the electrolytes are as a rule the same as in blood-serum, although sometimes rather divergent results have been found. The concentration of the electrolytes shows according to BODON,[5] like the blood-serum, much less variation than the total concentration. The alkalinity determined by titration is about the same in transudates and exudates, and is equal to that of the blood-serum. The determination of the HO ion concentration has shown that the transudates and exudates in this regard are about as neutral as the blood-serum (BODON).

As above stated, irrespective of the varying number of form-elements contained in the different transudates, the quantity of protein is the most characteristic chemical distinction in the composition of the various transudates; therefore a quantitative analysis is of importance only in so far as it considers the quantity of protein. On this account, in the

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[1] Pickardt, Berl. klin. Wochenschr., 1897. See also Rotmann, Münch. med. Wochenschr., 1898; Neuberg and Strauss, Zeitschr. f. physiol. Chem., **36**.

[2] Zeitschr. f. Biologie, **32**.

[3] Zeitschr. f. physiol. Chem., **13**.

[4] Zeitschr. f. Heilkunde, **25**.

[5] Pflüger's Arch., **104**, where the literature on this subject may be found.

following, relative to the quantitative composition, chief stress will be put on the quantity of protein.

Pericardial Fluid. The quantity of this fluid is, even under physiological conditions, so large that a sufficient quantity for chemical investigation has been obtained (from persons who had been executed). This fluid is lemon-yellow in color, somewhat sticky, and yields more *fibrin* than other transudates. The amount of solids, according to the analyses performed by v. Gorup-Besanez, Wachsmuth, and Hoppe-Seyler,[1] is 37.5–44.9 p. m., and the amount of protein is 22.8–24.7 p. m. The analysis made by Hammarsten of a fresh pericardial fluid from a young man who had been executed yielded the following results, calculated in 1000 parts by weight:

Water	960.85		
Solids	39.15		
Proteins	28.60	Fibrin	0.31
		Globulin	5.95
		Albumin	22.34
Soluble salts	8.60	NaCl	7.28
Insoluble salts	0.15		
Extractive bodies	2.00		

Friend[2] found nearly the same composition for a pericardial fluid from a horse, with the exception that this liquid was relatively richer in globulin. The ordinary statement that pericardial fluids are richer in fibrinogen than other transudates is hardly based on sufficient proof. In a case of chylopericardium, which was probably due to the rupture of a chylous vessel or caused by a capillary exudation of chyle because of stoppage, Hasebroek[3] found in 1000 parts of the fluid 103.61 parts solids, 73.79 parts proteins, 10.77 parts fat, 3.34 parts cholesterin, 1.77 parts lecithin, and 9.34 parts salts.

The **pleural fluid** occurs under physiological conditions in such small quantities that a chemical analysis of it cannot be made. Under pathological conditions this fluid may show very variable properties. In certain cases it is nearly serous, in others again sero-fibrinous, and in others similar to pus. There is a corresponding variation in the specific gravity and the properties in general. If a pus-like exudate is kept enclosed for a long time in the pleural cavity, a more or less complete maceration and solution of the pus-corpuscles is found to take place. The ejected yellowish-brown or greenish fluid may then be as rich in solids as the blood-serum; and an abundant flocculent precipitate of a nucleoalbumin or nucleoprotein (the *pyrin* of early writers) may be obtained on

[1] v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 401; Wachsmuth, Virchow's Arch., 7; Hoppe-Seyler, Physiol. Chem., 605.

[2] Halliburton, Text-book of Chem. Physiol., etc., London, 1891.

[3] Zeitschr. f. physiol. Chem., 12.

the addition of acetic acid. This precipitate is soluble with difficulty in an excess of acetic acid.

Numerous analyses, by many investigators,[1] of the quantitative composition of pleural fluids under pathological conditions have been published. From these analyses we learn that in hydrothorax the specific gravity is lower and the quantity of protein less than in pleuritis. In the first case the specific gravity is generally less than 1.015, and the quantity of protein 10–30 p. m. In acute pleuritis the specific gravity is generally higher than 1.020, and the quantity of protein 30–65 p. m. The quantity of fibrinogen, which in hydrothorax is about 0.1 p. m., may amount to more than 1 p. m. in pleuritis. In pleurisy with an abundant accumulation of pus, the specific gravity may rise even to 1.030, according to the observations of HAMMARSTEN. The quantity of solids is often 60–70 p. m., and may be even more than 90–100 p. m. (HAMMARSTEN). Mucoid substances have also been detected in pleural fluids by PAIJKULL. Cases of chylous pleurisy are also known; in such a case MÉHU [2] found 17.93 p. m. fat and cholesterin in the fluid.

The quantity of **peritoneal fluid** is very small under physiological conditions. The investigations refer only to the fluid under diseased conditions (*ascitic fluid*). The color, transparency, and consistency of these may vary greatly.

In cachectic conditions or a hydræmic condition of the blood the fluid has little color, is milky, opalescent, watery, does not coagulate spontaneously, has a very low specific gravity, 1.005–1.010–1.015, and is nearly free from form elements. The ascitic fluid in portal stagnation, or in general venous congestion, has a low specific gravity and ordinarily less than 20 p. m. protein, although in certain cases the quantity of protein may rise to 35 p. m. In carcinomatous peritonitis it may have a cloudy, dirty-gray appearance, due to its richness in form-elements of various kinds. The specific gravity is then higher, the quantity of solids greater, and it often coagulates spontaneously. In inflammatory processes it is straw- or lemon-yellow in color, somewhat cloudy or reddish, due to leucocytes and red blood-corpuscles, and from great richness in leucocytes it may appear more like pus. It coagulates spontaneously and may be relatively richer in solids. It contains regularly 30 p. m. or more protein (although exceptions with less protein occur), and may have a specific gravity of 1.030 or above. On account of the rupture of a chylous vessel, the ascitic fluid may be rich in very finely emulsified fat (CHYLOUS ASCITES). In such cases 3.86–10.30 p. m. fat has been

[1] See the works of Méhu, Runeberg, F. Hoffmann, Reuss, all of which are cited in Bernheim's paper in Virchow's Arch., **131**, 274. See also Paijkull, l. c., and Halliburton's Text-book, 346; Joachim, l. c.

[2] Arch. gén. de méd., 1886, **2**, cited from Maly's Jahresber., **16**.

found in the ascitic fluid (GUINOCHET, HAY [1]), and even 17–43 p. m. has been found by MINKOWSKI.

As first shown by GROSS, an ascitic fluid may have a chylous appearance without the presence of fat, i.e., pseudochylous. The cause of the chylous properties of a transudate is not known, although numerous investigators, such as GROSS, BERNERT, MOSSE, and STRAUSS, have studied the subject; several observations, however, seem to show that it is connected with the amount of lecithin contained therein. In a case investigated by H. WOLFF [2] the oleic-acid ester of cholesterin was combined either chemically or molecularly with the euglobulin.

By admixture of ascitic fluid with the fluid from an ovarian cyst the former may sometimes contain pseudomucin (see Chapter XIII). There are also cases in which the ascitic fluid contains mucoids which may be precipitated by alcohol after removal of the proteins by coagulation at boiling temperature. Such mucoids, which yield a reducing substance on boiling with acids, have been found by HAMMARSTEN in tuberculous peritonitis and in cirrhosis hepatis syphilitica in men. According to the investigations of PAIJKULL, these substances seem to occur often and perhaps habitually in the ascitic fluids.

As the quantity of protein in ascitic fluids is dependent upon the same factors as in other transudates and exudates, it is sufficient to give the following example of the composition, taken from BERNHEIM'S [3] treatise. The results are expressed in 1000 parts of the fluid:

	Max.	Min.	Mean.
Cirrhosis of the liver	34.5	5.6	9.69—21.06
Bright's disease	16.11	10.10	5.6 —10.36
Tuberculous and idiopathic peritonitis	55.8	18.72	30.7 —37.95
Carcinomatous peritonitis	54.20	27.00	35.1 —58.96

JOACHIM found the highest relative globulin amounts and lowest albumin percentages in cirrhosis; in carcinoma, on the contrary, the lowest globulin and the highest albumin. The values in cardiac stagnation stand between the cirrhosis and carcinoma percentages.

Urea has also been found in ascitic fluids, sometimes only as traces, sometimes in larger quantities (4 p. m. in albuminuria), also *uric acid, allantoin* in cirrhosis of the liver (MOSCATELLI), *xanthine, creatine, cholesterin, sugar, diastatic* and *proteolytic enzymes,* and according to HAMBURGER [4] also a *lipase.*

Hydrocele and Spermatocele Fluids. These fluids differ essentially from each other in various ways. The hydrocele fluids are generally colored light or dark yellow, sometimes brownish with a shade of green.

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[1] Guinochet, see Strauss, Arch. de Physiol., 18. See Maly's Jahresber., 16, 475.

[2] Gross, Arch. f. exp. Path. u. Pharm., 44; Bernert, *ibid.*, 49; Mosse, Leyden's Festschrift, 1901; Strauss, cited in Biochem. Centralbl., 1, 437; Wolff, Hofmeister's Beiträge, 5.

[3] l. c. As it was impossible to derive mean figures from those given by Bernheim, the author has given the maximum and minimum of the averages given by him.

[4] Arch. f. (Anat. u.) Physiol., 1900, 433.

They have a relatively higher specific gravity, 1.016–1.026, with a variable but generally higher amount of solids, an average of 60 p. m. They sometimes coagulate spontaneously, sometimes only after the addition of fibrin ferment or blood. They contain *leucocytes* as chief form-elements. Sometimes they contain smaller or larger amounts of *cholesterin crystals*.

The spermatocele fluids, on the contrary, are as a rule colorless, thin, and cloudy like water mixed with milk. They sometimes have an acid reaction. They have a lower specific gravity, 1.006–1.010, a lower amount of solids—an average of about 13 p. m.—and do not coagulate either spontaneously or after the addition of blood. They are, as a rule, poor in protein and contain *spermatozoa*, *cell-detritus*, and *fat-globules* as form constituents. To show the unequal composition of these two kinds of fluids we will give the average results (calculated in parts per 1000 parts of the fluid) of seventeen analyses of hydrocele fluids and four of spermatocele fluids made by HAMMARSTEN.[1]

	Hydrocele.	Spermatocele.
Water	938.85	986.83
Solids	61.15	12.17
Fibrin	0.59	.....
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether extractive bodies	4.02	
Soluble salts	8.60	10.76
Insoluble salts	0.66	

In the hydrocele fluid traces of *urea* and a reducing substance have been found, and in a few cases also *succinic acid* and *inosite*. A hydrocele fluid may, according to DEVILLARD,[2] sometimes contain paralbumin or metalbumin (?). Cases of chylous hydrocele are also known.

Cerebrospinal Fluid. The cerebrospinal fluid is thin, water-clear, of low specific gravity, 1.007–1.008. The spina bifida fluid is very poor in solids, 8–10 p. m. with only 0.19–1.6 p. m. protein. The fluid of chronic hydrocephalus is somewhat richer in solids (13–19 p. m.) and proteins. The amount of protein in the cerebrospinal fluid seems to be rather variable under diseased conditions and FRENKEL-HEIDEN[3] found 0.875–3 p. m. protein in the lumbar fluid in progressive paralysis and 0.7–2.8 p. m. protein in tuberculous meningitis. According to HALLIBURTON the protein of the cerebrospinal fluid is a mixture of *globulin* and *proteose;* occasionally some peptone occurs, and more rarely, in special cases, seralbumin appears. The conclusions of HALLIBURTON on the occurrence of *proteose* do not coincide with the observations of other investigators (PANZER, SALKOWSKI[4]). In general paralysis HALLI-

[1] Upsala Läkaref. Förh., **14**, and Maly's Jahresber., 8, 347.

[2] Bull. Soc. chim., **42**, 617.

[3] Bioch. Zeitschr., **2**.

[4] Halliburton's Text-book; Panzer, Wien. klin. Wochenschr., 1899; Salkowski, Jaffé Festschrift, 265.

BURTON and MOTT obtained a *nucleoprotein* in the cerebrospinal fluid. *Choline* occurs in several diseases, as in general paralysis, brain-tumors, tabes dorsalis, and epilepsy (HALLIBURTON and MOTT, DONATH, ROSENHEIM [1]). *Dextrose*, or at least a fermentable sugar, occurs habitually in the cerebrospinal fluid, while the claims of HALLIBURTON as to the occurrence of a substance similar to pyrocatechin could not be substantiated by NAWRATZKI,[2] and hence this substance does not exist in all cerebrospinal fluids. *Urea* occurs in cerebrospinal fluids, but not always. In the cases investigated by FRENKEL-HEIDEN indeed all the rest-nitrogen occurred as urea and the urea-nitrogen varied in different pathological cases between 0.196–1.12 p. m. *Lactic Acid* has been found by LEHNDORFF and BAUMGARTEN [3] in many pathological cases. The variable relation between potassium and sodium is probably due, according to SALKOWSKI,[4] to the absence or presence of fever during the formation of the exudate; the amount of potassium is high in the acute cases and low in the chronic ones. According to LANDAU and HALPERN [5] a certain antagonism seems to exist between nitrogen and sodium chloride, as the highest results of the first correspond to the lowest results of the other. According to CAVAZZANI,[6] who has especially studied the cerebrospinal fluids, the alkalinity of these fluids is considerably less than that of the blood and independent of this last fluid. For this and several other reasons CAVAZZANI draws the conclusion that the cerebrospinal fluid is formed by a true secretory process.

Aqueous Humor. This fluid is clear, alkaline toward litmus, and has a specific gravity of 1.003–1.009. The amount of solids is on an average 13 p. m., and the amount of proteins only 0.8–1.02 p. m. The protein consists of *seralbumin* and *globulin* and very little *fibrinogen* and *mucin*. According to GRUENHAGEN it contains *paralactic acid*, another dextrogyrate substance, and a *reducing body* which is unlike dextrose or dextrin. PAUTZ [7] found *urea* and *sugar* in the aqueous humor of oxen.

Blister-fluid. The content of blisters caused by burns, and of vesicatory blisters and the blisters of the *pemphigus chronicus*, is generally a fluid rich in solids and proteins (40–65 p. m.). This is especially true of the

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[1] Halliburton and Mott, Phil. Transact. Roy. Soc. London, Series B, **191**; Donath, Zeitschr. f. physiol. Chem., **39** and **42**; see also Mansfield, *ibid.*, **42**; Rosenheim, Journ. of Physiol., **35**.

[2] Zeitschr. f. physiol. Chem., **23**. See also Rossi, *ibid.*, **39** (literature).

[3] Zeitschr. f. exp. Path. u. Therap., **4** (literature).

[4] See Salkowski, l. c. New quantitative analyses of cerebrospinal and hydrocephalus fluids may be found in the cited works of Nawratzki, Panzer, and Salkowski.

[5] Bioch. Zeitschr., **9**.

[6] See Maly's Jahresber., **22**, 346, and Centralbl. f. Physiol., **15**, 216.

[7] Gruenhagen, Pflüger's Arch., **43**; Pautz, Zeitschr. f. Biologie, **31**.

contents of vesicatory blisters. In a burn-blister K. MÖRNER[1] found 50.31 p. m. proteins, among which were 13.59 p. m. globulin and 0.11 p. m. fibrin. The fluid contains a substance which reduces copper oxide, but no pyrocatechin. The fluid of the pemphigus is alkaline in reaction. A wound secretion collected by LIEBLEIN[2] under aseptic conditions was alkaline in reaction, and contained less protein than the blood-serum. It formed a slight fibrin clot, and contained proteoses only at first or at the beginning of the abscess formation. As the wound healed, the relation between the globulin and albumin changed, and on the third day of the healing the quantity of albumin was at least nine-tenths of the total protein.

The fluid of **subcutaneous œdema.** This is, as a rule, very poor in solids, purely serous, does not contain fibrinogen, and has a specific gravity of 1.005–1.013. The quantity of proteins is in most cases lower than 10 p. m.,—according to HOFFMANN 1–8 p. m.,—and in serious affections of the kidneys, generally with amyloid degeneration, less than 1 p. m. has been shown (HOFFMANN[3]). The œdematous fluid also habitually contains *urea*, 1–2 p. m., and *sugar*.

The FLUID OF THE ECHINOCOCCUS cyst is related to the transudates, and is poor in proteins. It is thin and colorless, and has a specific gravity of 1.005–1.015. The quantity of solids is 14–20 p. m. The chemical constituents are *sugar* (2.5 p. m.), *inosite*, traces of *urea*, *creatine*, *succinic acid*, and salts (8.3–9.7 p. m.). Proteins are found only in traces, and then only after an inflammatory irritation. In the last-mentioned case 7 p. m. proteins have been found in the fluid.

The Synovial Fluid and Fluid in Synovial Cavities around Joints, etc. The synovia is hardly a transudate, but it is often discussed in an appendix to the transudates.

The synovia is an alkaline, sticky, fibrous, yellowish fluid which is cloudy, from the presence of cell-nuclei and the remains of destroyed cells, but is also sometimes clear. Besides *proteins* and salts, it also contains a mucin substance, *synoviamucin* (v. HOLST[4]). In pathological synovia HAMMARSTEN found a mucin-like substance which is not mucin. It behaves like a nucleoalbumin or a nucleoprotein, and gives no reducing substance on boiling with acids. SALKOWSKI[5] also found a mucin-like substance in a pathological synovial fluid, which was neither mucin nor nucleoalbumin. He called the substance *synovin*.

The composition of synovia is not constant, but is different in rest and in motion. In the last-mentioned case the quantity of fluid is less,

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[1] Skand. Arch. f. Physiol., **5**.

[2] Habilitationsschrift Prag, 1902, printed by H. Laupp, Tübingen.

[3] Deutsch. Arch. f. klin. Med., **44**.

[4] Zeitschr. f. physiol. Chem., **43**.

[5] Hammarsten, Maly's Jahresber., **12**; Salkowski, Virchow's Arch., **131**.

but the amount of the mucin-like body, of proteins, and of the extractive bodies is greater, while the quantity of salts is diminished. This may be seen from the following analyses by FRERICHS.[1] The figures represent parts per 1000.

	I. Synovia from a Stall-fed Ox.	II. Synovia from a Field-fed Ox.
Water	969.9	948.5
Solids	30.1	51.5
Mucin-like body	2.4	5.6
Albumin and extractives	15.7	35.1
Fat	0.6	0.7
Salts	11.3	9.9

The synovia of new-born babes corresponds to that of resting animals. The fluid of the bursæ mucosæ, as also the fluid in the synovial cavities around joints, etc., is similar to synovia from a qualitative standpoint.

III. PUS.

Pus is a yellowish-gray or yellowish-green, creamy mass of a faint odor and an unsavory, sweetish taste. It consists of a fluid, the *pus-serum*, in which solid particles, the *pus-cells*, swim. The number of these cells varies so considerably that the pus may at one time be thin and at another time so thick that it scarcely contains a drop of serum. The specific gravity, therefore, may also greatly vary, namely, between 1.020 and 1.040, but ordinarily it is 1.031–1.033. The reaction of fresh pus is generally alkaline, but it may become neutral or acid from a decomposition in which fatty acids, glycerophosphoric acid, and also lactic acid are formed. It may become strongly alkaline when putrefaction occurs with the formation of ammonia.

In the chemical investigation of pus, the pus-serum and the pus-corpuscles must be studied separately.

Pus-serum. Pus does not coagulate spontaneously nor after the addition of defibrinated blood. The fluid in which the pus-corpuscles are suspended is not to be compared with the blood-plasma, but rather with the serum. The pus-serum is pale yellow, yellowish green, or brownish yellow, and has an alkaline reaction toward litmus. It contains, for the most part, the same constituents as the blood-serum; but sometimes besides these—when, for instance, the pus has remained in the body for a long time—it contains a nucleoalbumin or a nucleoprotein which is precipitated by acetic acid and is soluble with great difficulty in an excess of the acid (*pyin* of the earlier authors). This nucleoalbumin seems to be formed from the hyaline substance of the pus-cells by maceration. The pus-serum contains, moreover, at least in many cases, no fibrin

[1] Wagner's Handwörterbuch, 3, Abt. 41, 63.

ferment. According to the analyses of HOPPE-SEYLER [1] the pus-serum contains in 1000 parts:

	I.	II.
Water	913.70	905.65
Solids	86.30	94.35
Proteins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcohol extractives	1.52	0.73
Water extractives	11.53	6.92
Inorganic salts	7.73	7.77

The ash of pus-serum has the following composition, calculated to 1000 parts of the serum.

	I.	II.
NaCl	5.22	5.39
Na_2SO_4	0.40	0.31
Na_2HPO_4	0.98	0.46
Na_2CO_3	0.49	1.13
$Ca_3(PO_4)_2$	0.49	0.31
$Mg_3(PO_4)_2$	0.19	0.12
PO_4 (in excess)	....	0.05

The **pus-corpuscles** are generally thought to consist chiefly of emigrated white blood-corpuscles, and their chemical properties have therefore been given in discussing these. The molecular granules, fat-globules, and red blood-corpuscles are considered rather as casual form-elements.

The pus-cells may be separated from the serum by centrifugal force, or by decantation directly or after dilution with a solution of sodium sulphate in water (1 vol. saturated sodium-sulphate solution and 9 vols. water), and then washed by this same solution in the same manner as the blood-corpuscles.

The chief constituents of the pus-corpuscles are proteins, of which the largest portion seems to be a nucleoprotein which is insoluble in water and which expands into a tough, slimy mass when treated with a 10-per cent common-salt solution. This protein substance, which is soluble in alkali but is quickly changed thereby, is called ROVIDA'S *hyaline substance*, and the property of the pus of being converted into a slime-like mass by a solution of common salt depends on this substance. Besides this substance, to which the nucleoprotein of the pus-cells investigated by STRADA [2] seems to stand in close relation, we also have a *globulin* which coagulates at 48–49° C., as well as *serglobulin* (?), *seralbumin*, a substance similar to coagulated protein (MIESCHER), and lastly *peptone*

[1] Med.-chem. Untersuch., 490.

[2] Bioch. Zeitschr., **16**.

or *proteose* (HOFMEISTER[1]). It is very remarkable that no nucleohistone or histone has been detected in the pus-cells, although histone occurs in the cells of the lymph glands.

There are also found in the protoplasm of the pus-cells, besides the proteins, *lecithin, cholesterin, glucothionic acid,*[2] *purine bodies, fat,* and *soaps.* HOPPE-SEYLER has found *cerebrin,* a decomposition product of a protagon-like substance, in pus (see Chapter XII). KOSSEL and FREYTAG[3] have isolated from pus two substances, *pyosin* and *pyogenin,* which belong to the cerebrin group (see Chapter XII). HOPPE-SEYLER[4] claims that *glycogen* appears only in the living, contractile white blood-cells and not in the dead pus-corpuscles. Several other investigators have nevertheless found glycogen in pus. The cell-nucleus contains *nuclein* and *nucleoproteins.*

In regard to the occurrence of *enzymes* in the pus-cells it must be remarked that neither thrombin nor prothrombin is found therein, although these bodies are generally considered as being derived from the leucocytes, and also obtainable from the thymus leucocytes. The occurrence in the pus-cells, besides catalases and oxidases, of a proteolytic enzyme, is of great interest. It is not only important for the intracellular digestion and for the amount of proteoses in the pus-cell, but also for the solution of the fibrin clot and pneumonic infiltrations (FR. MÜLLER, O. SIMON[5]).

The *mineral constituents* of the pus-corpuscles are potassium, sodium, calcium, magnesium, and iron. A part of the alkalies exists as chlorides, and the remainder, as well as the chief part of the other bases, exists as phosphates.

The quantitative composition of the pus-cells from the analyses of HOPPE-SEYLER is as follows, in parts per 1000 of the dried substance:

	I.		II.
Proteins	137.62	685.85	673.69
Nuclein	342.57		
Insoluble bodies	205.66		
Lecithin	143.83		75.64
Fat			75.00
Cholesterin	74.00		72.83
Cerebrin	51.99		102.84
Extractive bodies	44.33		

[1] Miescher in Hoppe-Seyler's Med.-chem. Untersuch., 441; Hofmeister, Zeitschr. f. physiol. Chem., **4**.

[2] Mandel and Levene, Bioch. Zeitschr., **4**.

[3] Zeitschr. f. physiol. Chem., **17**, 452.

[4] Hoppe-Seyler, Physiol. Chem., 790.

[5] Fr. Müller, Verhandl. Nat. Gesellsch. zu Basel, 1901; O. Simon, Deutsch. Arch. f. klin. Med., **70**.

MINERAL SUBSTANCES IN 1000 PARTS OF THE DRIED SUBSTANCE

NaCl	4.34
$Ca_3(PO_4)_2$	2.05
$Mg_3(PO_4)_2$	1.13
$FePO_4$	1.06
PO_4	9.16
Na	0.68
K	Traces (?)

MIESCHER obtained other results for the alkali compounds, namely, potassium phosphate 12, sodium phosphate 6.1, earthy phosphate and iron phosphate 4.2, sodium chloride 1.4, and phosphoric acid combined with organic substances 3.14–2.03 p. m.

In pus from congested abscesses which has stagnated for some time there occur *peptone* (proteose), *leucine* and *tyrosine*, free *fatty acids* and *volatile fatty acids*, such as formic acid, butyric acid and valeric acid. There are also found *chrondrin* (?) and *glutin* (?), *urea*, *dextrose* (in diabetes), *bile-pigments*, and *bile-acids* (in catarrhal icterus).

As more specific but not constant constituents of the pus must be mentioned the following: *pyin*, which seems to be a nucleoprotein precipitable by acetic acid, and also *pyinic acid* and *chlorrhodinic acid*, which have been so little studied that they cannot be more fully treated here.

In many cases a blue, more rarely a green, color, has been observed in the pus. This depends on the presence of micro-organisms (*Bacillus pyocyaneus*). From such pus FORDOS and LÜCKE [1] have isolated a crystalline blue pigment, *pyocyanin*, and a yellow pigment, *pyoxanthose*, which is produced from the first by oxidation.

Appendix.

Lymphatic Glands, Spleen, etc.

The Lymphatic Glands. The cells of the lymphatic glands are found to contain the protein substances generally occurring in cells (Chapters I and VI). According to BANG [2] they also contain histone nucleates (*nucleohistone*), but in smaller amounts and of a different variety from the better-studied nucleohistone from the thymus gland. Proteoses occur as products of autolysis. By a lengthy autolysis of lymph glands REH [3] found ammonia, tyrosine, leucine (somewhat scanty), thymine, and uracil among the cleavage products. Besides the other ordinary tissue constituents, such as collagen, reticulin, elastin, and nuclein, there occur in the lymphatic glands also *cholesterin*, *fat*, *glycogen*, *sarcolactic acid*, *purine bases*, and *leucine*. In the inguinal glands of an old

[1] Fordos, Compt. rend., **51** and **56**; Lücke, Arch. f. klin. Chirurg., **3**; Boland, Centralbl. f. Bakt. u. Parasit., I, **25**.

[2] Studier over Nucleoproteider, Kristiania, 1902, and Hofmeister's Beiträge, **4**.

[3] Hofmeister's Beiträge, **3**.

woman OIDTMANN found 714.32 p. m. water, 284.5 p. m. organic and 1.16 p. m. inorganic substances. In the cells of the mesenteral lymphatic glands of oxen BANG [1] found 804.1 p. m. water, 195.9 p. m. solids, 137.9 total proteins, 6.9 p. m. histone nucleate, 10.6 p. m. nucleoprotein, 47.6 p. m. bodies soluble in alcohol, and 10.5 p. m. mineral constituents.

The Thymus. The cells of this gland are very rich in nuclein bodies and relatively poor in the ordinary proteins, but their nature has not been closely studied. The chief interest is attached to the nuclein substances. KOSSEL and LILIENFELD first prepared from the watery extract of the gland, by precipitating with acetic acid and then further purifying, a protein substance which has been generally called *nucleohistone*. By the action of dilute hydrochloric acid upon nucleohistone it splits, according to these investigators, into histone and leuconuclein. The leuconuclein is a true nuclein; hence it is a nucleic-acid compound with protein which is relatively poor in protein and rich in phosphorus. The more recent investigations of BANG, MALENGREAU, and HUISKAMP [2] upon nucleohistone all show that this nucleoprotein is not a unit substance, but a mixture of at least two bodies. The views of the investigators mentioned differ quite essentially from one another as to the nature of these bodies, but this is partly due to the different methods used by them and partly to the ready changeability of the substances in question.

Besides the real nucleohistone, B-nucleoalbumin of MALENGREAU, LILIENFELD'S histone contains a second nucleoprotein which BANG and HUISKAMP call simple nucleoprotein, while MALENGREAU designates it A-nucleoalbumin. This protein, which contains only about 1 per cent phosphorus and which is possibly identical with the nucleoprotein found by LILIENFELD in the thymus, yields a nuclein, but no free nucleic acid, on cleavage. As a second cleavage product it yields, according to MALENGREAU, the A-histone, which can be readily precipitated by magnesium and ammonium sulphates from the ordinary B-histone of the thymus gland. The occurrence of A-histone in the gland has been verified by BANG, and according to BANG and HUISKAMP the A-histone is not derived from the nucleoprotein, as these investigators claim that it yields no histone. According to BANG the nucleoprotein yields only an albuminate, besides the nuclein, as cleavage products.

The true nucleohistone, which is much richer in phosphorus (the calcium salt containing, according to BANG, on an average 5.23 per cent P), yields ordinary histone as one cleavage product and free nucleic acid

[1] l. c.

[2] Lilienfeld, Zeitschr. f. physiol. Chem., **18**; Kossel, *ibid.*, **30** and **31**; Bang, *ibid.*, **30** and **31**. See also Arch. f. Math. og Naturvidenskab, **25**, Kristiania, 1902, and Hofmeister's Beiträge, **1** and **4**; Malengreau, La Cellule, **17** and **19**; Huiskamp, Zeitschr. f. physiol. Chem., **32**, **34**, and **39**.

as the other, according to the unanimous opinion of the above-mentioned investigators. According to BANG, whose statements on this point have been substantiated by MALENGREAU, it splits on saturating with NaCl into nucleic acid and histone without yielding any other protein. On this account BANG does not consider this body as nucleohistone in the ordinary sense, i.e., not as a nucleoprotein, but as a histone nucleate. The nucleohistone behaves like an acid, whose salts, especially the calcium salt, have been closely studied by HUISKAMP. On the electrolysis of a solution of alkali nucleohistone in water HUISKAMP also found that the nucleohistone collected in traces at the anode, and that the sodium compound is therefore ionized in the solution. The nucleic acid-calcium histone-compound has been prepared, it seems, in a pure state by BANG, and he found the following average composition: C 43.69; H 5.60; N 16.87; S 0.47; P 5.23; Ca 1.71 per cent. The question as to what compound contains the A-histone remains to be investigated.

The nucleohistone prepared by HUISKAMP'S method of precipitating with $CaCl_2$ is, according to him, a mixture of two nucleohistones, of which one, the α-nucleohistone, contains 4.5 per cent phosphorus, and the other, β-nucleohistone, contains, on the contrary, only in round numbers 3 per cent phosphorus.[1] As the two nucleohistones are poorer in phosphorus than the nucleic acid-histone compound analyzed by BANG, and as HUISKAMP on cleavage of his preparation did not, like BANG and MALENGREAU, obtain pure nucleic acid, it is still a question whether HUISKAMP was working with sufficiently pure substances.

In regard to the methods used by the above investigators in the isolation of the bodies in question we must refer to the original publications.

In connection with the so-called nucleohistone, attention must be called to *tissue fibrinogen* and *cell fibrinogen*, which are compound proteins, and are claimed by certain investigators to stand in close relation to the coagulation of the blood. These may be in part nucleoproteins and in part also nucleohistones. To this same group belong also the important cell constituents described by ALEX. SCHMIDT[2] and called *cytoglobin* and *preglobulin*. The cytoglobin, which is soluble in water, may be considered as the alkali compound of preglobulin. The residue of the cells left after complete extraction with alcohol, water, and salt solution has been called *cytin* by ALEX. SCHMIDT.

Besides the above-mentioned and the ordinary bodies belonging to the connective-tissue group, small quantities of *fat, leucine, succinic acid, lactic acid, sugar,* and traces of *iodothyrin* are present. According to GAUTIER[3] *arsenic* also occurs in very small amounts, and no doubt here as well as in other organs it is related to the nuclein substances. The richness in nuclein bodies explains the occurrence of large quantities of *purine bases,* chiefly *adenine,* whose quantity, according to KOSSEL and SCHINDLER,[4] is 1.79 p. m. in the fresh organ and 19.19 p. m. in the

[1] Zeitschr. f. physiol. Chem., **39**.
[2] See foot-note 1, p. 295.
[3] Compt. rend., **129**.
[4] Zeitschr. f. physiol. Chem., **13**.

dry substance, and *guanine*. The bodies *thymine* and (*uracil ?*) obtained, besides lysine and ammonia, by KUTSCHER, as products of autodigestion of the gland, probably have a similar origin. LILIENFELD[1] found *inosite* and *protagon* in the cells of the thymus. Among the enzymes, besides *arginase*, *guanase*, and *adenase*, we must especially mention the enzyme studied by JONES,[2] which acts like a *nuclease*, splitting off phosphoric acid and purine bases, from the nucleoproteins. This enzyme, contrary to trypsin, acts best in acid liquids, and is readily destroyed by alkalies at body temperature. The quantitative composition of the lymphocytes of the thymus of a calf is, according to LILIENFELD'S analysis, as follows. The results are given in 1000 parts of the dried substance:

Proteids	17.7
Leuconuclein	687.9
Histone	86.7
Lecithin	75.1
Fat	40.2
Cholesterin	44.0
Glycogen	8.0

The dried substance of the leucocytes amounted to an average of 114.9 p. m. Potassium and phosphoric acid are prominent mineral constituents. LILIENFELD found KH_2PO_4 among the bodies soluble in alcohol.

Attention must be called to the analyses of BANG,[3] which show that the thymus contains about the same quantity of nucleoprotein, but about five times as much histone nucleate as the lymphatic glands—calculated in both cases upon the same amount of dry substance. OIDTMANN[4] found 807.06 p. m. water, 192.74 p. m. organic and 0.2 p. m. inorganic substances in the gland of a child two weeks old.

The Spleen. The pulp of the spleen cannot be freed from blood. The mass which is separated from the spleen capsule and the structural tissue by pressure, and which ordinarily serves as material for chemical investigations is, therefore a mixture of blood and spleen constituents. For this reason the proteins of the spleen are little known. The nucleoprotein isolated by LEVENE and MANDEL[5] is to be considered as a true spleen constituent, and this nucleoprotein yields 25 per cent glutamic acid on hydrolysis. Histone has not been directly detected in the spleen; but its presence is to be admitted because KRASNOSSELSKY[6] was able to isolate a histone-peptone as sulphate from the spleen. The *ferruginous*

[1] Kutscher, Zeitschr. f. physiol. Chem., **34**; Lilienfeld, *ibid.*, **18**.

[2] Zeitschr. f. physiol. Chem., **41**.

[3] l. c., Arch. f. Math., etc.

[4] Cited from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., p. 732.

[5] Bioch. Zeitschr., **5**.

[6] Zeitschr. f. physiol. Chem., **49**.

albuminate has been considered as a spleen constituent for a long time, and especially also a protein substance which does not coagulate on boiling and which is precipitated by acetic acid and yields an ash containing much phosphoric acid and iron oxide.[1]

The pulp of the spleen, when fresh, has an alkaline reaction, but quickly turns acid, due partly to the formation of free *paralactic acid* and partly perhaps to *glycerophosphoric acid.* Besides these two acids there are found in the spleen also *volatile fatty acids,* as formic, acetic, and butyric acids, as well as *succinic acid, neutral fats, cholesterin,* traces of *leucine, inosite* (in ox-spleen), *scyllite,* a body related to inosite (in the spleen of plagiostoma), *glycogen* (in dog-spleen), *uric acid, purine bases,* and *jecorin.* LEVENE found in the spleen a *glucothionic acid,* i.e., an acid which is related to chondroitin-sulphuric acid but not identical therewith, and which gives a beautiful violet coloration with orcin and hydrochloric acid. The question whether this glucothionic acid originates from the above-mentioned nucleoprotein or from the mucoid substance has not been decided (LEVENE and MANDEL). In regard to the question whether this acid is a unit body or not we refer to the work of MANDEL and NEUBERG and LEVENE and JACOBS.[2]

Many enzymes are found in the spleen, and certain of these are of special interest. To these belong the uric-acid-forming enzyme, the *xanthine oxidase* (BURIAN), which occurs in the spleen of oxen and horses, but not in man, dogs, and pigs (SCHITTENHELM), and which transforms the oxypurines, hypoxanthine, and xanthine into uric acid; also the hydrolytically active deamidizing enzymes *guanase* and *adenase* (LEVENE, SCHITTENHELM, JONES and PARTRIDGE, JONES and WINTERNITZ), by the first of which the guanine is transformed into xanthine, and by the latter the adenine into hypoxanthine. The guanase also occurs in the spleen of the ox and horse, but not (JONES), or only in small amounts (SCHITTENHELM), in the pig-spleen.[3] The spleen also contains two enzymes, *lienases,* as shown by HEDIN (and ROWLAND), one of which, the α-lienase, acts chiefly in alkaline solution, while the other, β-lienase, is active only in acid reaction. These enzymes, which without doubt stand in close relation to the leucocytes, not only act autolytically upon the proteins of the spleen, but they also dissolve fibrin and coagulated blood-serum. In the autolysis of the spleen LEATHES found among the cleavage products, proteoses, lysine, arginine, histidine, leucine, amino-valeric acid, aspartic acid, and tryptophane. SCHUMM[4] found, in the

[1] See v. Gorup-Besanez, Lehrbuch, 4. Aufl., p. 717.

[2] Levene, Zeitschr. f. physiol. Chem., **37**; Levene and Mandel, *ibid.*, **45** and **47**; Mandel and Neuberg, Bioch. Zeitschr., **13**; Levene, *ibid.*, **16**; Neuberg, *ibid.*, **16**.

[3] See chapter XV for the literature.

[4] Hedin and Rowland, Zeitschr. f. physiol. Chem., **32**, and Hedin, Journ. of Physiol.,

autolysis of a leucæmic spleen, besides leucine and tyrosine, relatively large quantities of ammonia, also *r*-alanine, histidine, and lysine (but no arginine), guanine, xanthine, hypoxanthine, thymine, and *p*-lactic acid. The autolysis of the leucæmic spleen was much more extensive than the normal.

Among the constituents of the spleen the *deposit rich in iron*, which consists of ferruginous granules or conglomerate masses of them, and which is derived from a transformation of the red blood-corpuscles, is of special interest. It was closely studied by NASSE. This deposit does not occur to the same extent in the spleen of all animals. It is found especially abundant in the spleen of the horse. NASSE [1] on analyzing the grains (from the spleen of a horse) obtained 840–630 p. m. organic and 160–370 p. m. inorganic substances. These last consisted of 566–726 p. m. Fe_2O_3, 205–388 p. m. P_2O_5, and 57 p. m. earths. The organic substances consisted chiefly of proteins (660–800 p. m.), nuclein (52 p. m. maximum), a yellow coloring-matter, extractive bodies, fat cholesterin, and lecithin.

In regard to the *mineral constituents*, it is to be observed that in comparison with sodium and phosphoric acid the amount of potassium and chlorine is small. The amount of iron in new-born and young animals is small (LAPICQUE, KRÜGER, and PERNOU), in adults more appreciable, and in old animals sometimes very considerable. NASSE found nearly 50 p. m. iron in the dried pulp of the spleen of an old horse. GUILLEMONAT and LAPICQUE [2] have determined the iron in man. They find no regular increase with growth, but in most cases 0.17–0.39 p. m. (after subtracting the blood-iron) calculated on the fresh substance. A remarkably high amount of iron is not dependent upon old age, but is a residue from chronic diseases.

The quantitative analyses of the human spleen by OIDTMANN [3] give the following results: In men he found 750–694 p. m. water and 250–306 p. m. solids. In that of a woman he found 774.8 p. m. water and 225.2 p. m. solids. The quantity of inorganic bodies was in men 4.9–7.4 p. m., and in women 9.5 p. m.

In regard to the pathological processes going on in the spleen we must specially recall the abundant re-formation of leucocytes in leucæmia and the appearance of amyloid substance (see page 168).

30; and Hammarsten's Festschrift, 1906; Leathes, Journ. of Physiol., **28**; Schumm, Hofmeister's Beiträge, **3** and **7**.

[1] Maly's Jahresber., **19**, p. 315.

[2] Lapicque, *ibid.*, **20**; Lapicque and Guillemonat, Compt, rend. de soc. biol., **48**, and Arch. de Physiol. (5) **8**; Krüger and Pernou, Zeitschr. f. Biologie, **27**; Nasse, cited from Hoppe-Seyler, Physiol. Chem., 720.

[3] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., p. 719.

The physiological functions of the spleen are little known, with the exception of its importance in the formation of leucocytes. Some consider the spleen as an organ for the dissolution of the red blood-corpuscles, and the occurrence of the above-mentioned deposit rich in iron seems to confirm this view, but this iron could in part have another origin. ASHER and GROSSENBACHER found that the spleen is an organ for the iron metabolism, as they found in a splenectomized dog that the iron elimination was much greater than in a dog with its spleen. GROSSENBACHER and ZIMMERMANN [1] found that a splenectomized dog eliminates more iron than a normal dog even 10–11 months after the operation. This shows that no compensation occurs even after this long time. The spleen seems to be of little importance in taking up artificially introduced iron. The destruction of blood-corpuscles caused by pyrodine (acetylphenylhydrazine) increases the elimination of iron by normal dogs and also in splenectomized dogs, but in these latter to a much higher degree. The destruction of body substance produced experimentally by insufficient food or iron-free food causes a strongly increased elimination of iron and comparatively much stronger in splenectomized animals as compared with normal animals. This speaks in favor of ASHER'S view that the spleen is an organ which works up the iron set free in the destruction of the body material containing iron.

The spleen has also been claimed to play a certain part in digestion. This organ is said by SCHIFF, HERZEN, and others to be of importance in the production of trypsin in the pancreas. The investigations of HERZEN seem to confirm this relation, but the recent work of PRYM [2] has made the assumption doubtful.

An increase in the quantity of uric acid eliminated in splenic leucæmia has been observed by many investigators (see Chapter XV), while the reverse has been observed under the influence of quinine in large doses, which produces an enlargement of the spleen. These facts give a rather positive proof that there is a close relation between the spleen and the formation of uric acid. This relation has been studied by HORBACZEWSKI. He has shown that when the spleen-pulp and blood of calves are allowed to act on each other, under certain conditions and temperature, in the presence of air, large quantities of uric acid are formed. Under other conditions he obtained from the spleen-pulp only purine bases with very little or no uric acid. HORBACZEWSKI [3] has also shown that the uric acid originates from the nucleins of the spleen, which yield

[1] Asher and Grossenbacher, Centralbl. f. Physiol., **22**, 375, and Grossenbacher and Zimmermann, Bioch. Zeitschr., **17**.

[2] Schiff, cited by Herzen, Pflüger's Arch., **30**, 295, 308, and **84**, and Maly's Jahresber., **18**; Prym, Pflüger's Arch., **104** and **107**; see also Chapter IX.

[3] Monatshefte f. Chem., **10**, and Wien. Sitzungsber. Math. Nat. Klasse, **100**, Abt. 3.

uric acid or purine bases according to the experimental conditions. This behavior is explained by the above-mentioned investigations of BURIAN, SCHITTENHELM, JONES, and others on the enzymotic formation of uric-acid and the deamidization of the purine bodies, and a relation between the spleen and uric-acid formation is indisputable. Still we cannot say that the spleen shows a special relation to the uric-acid formation as compared with other organs (see Chapter XV).

The spleen has the same property as the liver of retaining foreign bodies, metals and metalloids.

The Thyroid Gland. The nature of the different protein substances occurring in the thyroid gland has not been sufficiently studied, but at present, through the researches of OSWALD, there are known at least two bodies which are constituents of the so-called secretion of the glands. One of these, *iodothyreoglobulin*, behaves like a globulin, while the other is a nucleoprotein (see also GOURLAY [1]). The iodine present in the gland occurs chiefly in the first body, while the arsenic, which has been shown to be a normal constituent by GAUTIER and BERTRAND,[2] seems to be related to the nuclein substances.

According to OSWALD the iodothyreoglobulin occurs only in those glands which contain colloid, while the colloid-free glands, the parenchymatous goitre, and the glands of the new-born contain thyreoglobulin free from iodine. The thyreoglobulin first becomes iodized into iodothyreoglobulin on passing from the follicle-cells. Besides these mentioned bodies *leucine, xanthine, hypoxanthine, choline,*[3] *iodothyrine, lactic* and *succinic acids* occur in the thyreoidea. OIDTMANN [4] found in the thyroid gland of an old woman 822.4 p. m. water, 176.6 p. m. organic and 0.9 p. m. inorganic substances. He found 772.1 p. m. water, 223.5 p. m. organic and 4.4 p. m. inorganic substances in an infant two weeks old.

In "STRUMA CYSTICA" HOPPE-SEYLER found hardly any protein in the smaller glandular vessels, but an excess of *mucin*, while in the larger he found a great deal of *protein*, 70–80 p. m.[5] *Cholesterin* is regularly found in such cysts, sometimes in such large quantities that the entire contents form a thick mass of cholesterin plates. Crystals of *calcium oxalate* also occur frequently. The contents of the struma cysts are sometimes of a brown color, due to decomposed coloring-matter, *methæmoglobin* (and hæmatin?). Bile-coloring matters have also been found in such cysts. (In regard to the *paralbumins* and *colloids* which have been found in struma cysts and colloid degeneration, see Chapter XIII).

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[1] Gourlay, Journ. of Physiol., **16**; Oswald, Zeitschr. f. physiol. Chem., **32**, and Biochem. Centralbl., **1**, 429.

[2] Gautier, Compt. rend., **129**. See also *ibid.*, **130**, **131**, **134**, **135**; Bertrand, *ibid.*, **134**, **135**.

[3] v. Fürth and Schwarz, Pflüger's Arch., **124**.

[4] l. c., 732.

[5] Physiol. Chem., p. 721.

Those substances which bear a close relation to the functions of the gland seem to be of special interest.

The complete extirpation, as also the pathological destruction, of the thyroid gland causes great disturbances, ending finally in death. In dogs, after the total extirpation, a disturbance of the nervous and muscular systems occurs, such as trembling and convulsions, and death generally supervenes shortly after, most often during such an attack. The researches of GLEY, VASSALE and GENERALI [1] upon various animals have shown that for the success of the operation it is of the greatest importance whether the parathyroids, discovered by SANDSTRÖM,[2] are removed at the same time or not. In herbivora (rabbits) because of the anatomical relations, the parathyroids are seldom extirpated in the operation of the removal of the thyroid, the tetany does not regularly occur and the disturbance in metabolism is most striking. If these glands are not extirpated in dogs the tetany also does not appear and the disturbances in metabolism occur. In human beings, after the removal of the gland by operation, different disturbances appear, such as nervous symptoms, diminished intelligence, dryness of the skin, falling out of the hair, and, on the whole, those symptoms which are included under the name cachexia thyreopriva, death coming gradually. Among these symptoms must be mentioned the peculiar slimy infiltration and exuberance of the connective tissue called myxœdema.

All these conditions indicate that the thyroids belong to those glands with internal secretion, so called endocrinogenic glands. The most convincing proof of this is the fact that the ordinary symptoms do not occur if a small piece of the gland is allowed to remain in the body, or even when a piece of the gland is transplanted in any part of the body. A further proof of practical importance is that the injurious results from removal of the thyroids can be counteracted by the introduction of artificial extracts of the thyroid gland into the body or by feeding with thyroid glands.

Of the disturbances in metabolism which occur on the extirpation or reduction of the thyroid function (athyreoidismus or hypothyreoidismus) we must especially mention the reduction in the protein catabolism which in a starving dog without thyroids may fall to about one-half of the starvation protein metabolism in a normal dog of the same size (FALTA and collaborators [3]). The reverse is observed when large quantities of the thyroid gland substance is fed, namely, a strong increase in the protein metabolism, besides certain other symptoms. BASEDOW'S disease

[1] Gley, Compt. rend. soc. biol., 1891, and Arch. de Physiol (5), **4**; Vassale and Generali, Arch. Ital. d. Biol., **25** and **26**.

[2] Upsala Läkaref. Förh., **15** (1880).

[3] Eppinger, Falta and Rudinger, Zeitschr. f. klin. Med., **66**.

is also considered as a form of hyperthyreoidismus which, by an increased activity of the glands, brings about an overproduction of the specific secretion.

It is impossible for the present to state anything about the kind of bodies here, having a specific action or anything about the importance of the bases found by certain investigators, such as S. Fränkel, Drechsel, and Kocher[1]; these bodies have not been characterized sufficiently. It seems proven that the specifically active substance is, as first shown by Notkin[2] and Oswald,[3] a protein substance: Notkin's *thyreoproteid*, Oswald's *thyreoglobulin*. This does not conflict with the views of Baumann and Roos that the active substance is *iodothyrin*, as this can be produced as a cleavage product from the iodothyreoglobulin. In fact Oswald[4] has found in the tryptic digestion of iodothyreoglobulin that a substance similar to iodothyrin is produced; for several reasons it seems that the action of the thyroid gland substance is not due to one substance, but to several.

Iodothyrin is considered by Baumann, who first showed that the thyroid contained iodine and who with Roos[5] proved the importance of this substance for the physiological activity of the gland, as the only active substance. By boiling the finely divided gland with dilute sulphuric acid Baumann obtained iodothyrin as an amorphous, brown mass, nearly insoluble in water but readily soluble in alkali and precipitated again by the addition of acid. The iodothyrin, which is not a unit body, has a variable content of iodine and is not a protein substance. According to v. Fürth and Schwarz it is probably a melanoid-like transformation product of the iodized protein of the gland produced by the action of the acid.

Thyreoglobulin or **iodothyreoglobulin** was obtained by Oswald from the watery extract of the gland by half saturating with ammonium sulphate. It has the properties of the globulins and with the exception of the iodine content it has about the same composition as the proteins. The amount of iodine varies: 0.46 per cent in pigs, 0.86 per cent in oxen, and 0.34 per

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[1] Fränkel, Wien. med. Blätter, 1895 and 1896; Drechsel and Kocher, Centralbl. f. Physiol., **9**, 705.

[2] Wien. med. Wochenschr., 1895, and Virchow's Arch., **144**, Suppl., 224.

[3] Zeitschr. f. physiol. Chem., **32**, and Bioch. Centralbl., **1**, 249.

[4] Arch. f. exp. Path. u. Pharm., **60**.

[5] In regard to this subject, see Baumann and Roos, Zeitschr. f. physiol. Chem., **21** and **22**; also Baumann, Münch. med. Wochenschr., 1896; Baumann and Goldmann, *ibid.*; Roos, *ibid.*; v. Fürth and Schwarz, Pflüger's Arch., **124**. An extensive review of the literature on the action of iodothyrin and the thyroid preparations can be found in Roos, Zeitschr. f. physiol. Chem., **22**, 18. In regard to their action in protein destruction and metabolism, see F. Voit, Zeitschr. f. Biologie, **35**; Schöndorff, Pflüger's Arch., **67**, and Anderson and Bergman, Skand. Arch. f. Physiol., **8**; Magnus-Levy, Zeitschr. f. klin. Med., **32**.

cent in man. In the iodothyreoglobulin of the ox, NÜRENBERG [1] found 0.59–0.86 per cent iodine and 1.83–2.0 per cent sulphur. In young animals, whose glands contain no iodine, the thyreoglobulin is iodine-free. Thyreoglobulin on taking up iodine is converted into iodothyreoglobulin. By introducing iodine salts the iodine content of the iodothyreoglobulin can be raised in living animals and thus the physiological activity increased (OSWALD). The amount of iodine in the gland is markedly dependent upon the food.

JOLIN [2] has examined a large number of thyroid glands from healthy and diseased persons (in SWEDEN), for their iodine content. In 28 children, ages varying between 1 and 10 years, he found an average of 0.28 p. m. iodine in the glands. In 108 normal glands above 10 years old or adults the iodine content varied with an average of 1.56 p. m. iodine. In glands from persons after using iodine preparations (34 cases) the iodine content was 2.56 p. m.

We cannot enter into a discussion as to the various hypotheses and theories in regard to the mode of action of the constituents of the thyroids.[3]

The Adrenal Bodies. Besides proteins, substances of the connective tissue, and salts, there occur in the suprarenal capsule *inosite*, purine bases, especially *xanthine* (OKER-BLOM), a protagon-like substance (ORGLER), relatively considerable *lecithin* and *choline*, and *glycerophosphoric acid*, which are probably decomposition products of the lecithin. The earlier accounts of the occurrence of benzoic acid, hippuric acid, and bile-acids are, on the contrary, doubtful, and are not substantiated by recent investigations (STADELMANN). Earlier investigators, like VULPIAN and ARNOLD,[4] have found in the medulla a *chromogen* which has been considered as connected with the abnormal pigmentation of the skin in Addison's disease. This chromogen, which is transformed by air, light, alkalies, iodine, and other bodies into a red pigment, seems, on the contrary, to be related to the substance *adrenalin*, of the gland which produces an increase in the blood-pressure. Choline has been shown to have a reverse effect upon this blood pressure raising action, and LOHMANN [5] has shown that it is formed in the cortical substance of the adrenals. That the watery extract of the adrenals has a blood-pressure raising action was shown by OLIVER and SCHÄFER, CYBULSKI and SZYMONOWICZ.[6] The substance which is here active was formerly called sphyg-

[1] Bioch. Zeitschr., **16**.

[2] Hammarsten's Festschr., 1906.

[3] A summary of the thyroid literature may be found in Maly's Jahresber., **24** and **25**. See also the works of Blum and Oswald, cited by Oswald in Biochem. Centralbl., **1**, 249.

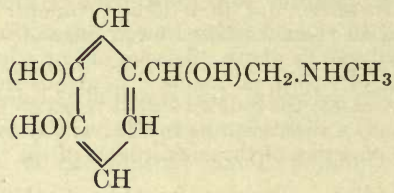
[4] Oker-Blom, Zeitschr. f. physiol. Chem., **28**; Stadelmann, *ibid.*, **18**, which also contains the literature on this subject; Orgler, Salkowski's Festschrift, 1904.

[5] Centralbl. f. Physiol., **21**, and Pflüger's Arch., **118**.

[6] Oliver and Schäfer, Proceed. of Physiol. Soc., London, 1895. Further literature on the function of the adrenals may be found in Szymonowicz, Pflüger's Arch., **64**.

mogenin and has also other actions besides bringing about a marked increase in blood-pressure by the strong contraction of the muscles of the periphery vessels; for instance, it can bring about glycosuria and mydriasis. This body has been chemically investigated by several experimenters such as v. FÜRTH, ABEL, TAKAMINE, ALDRICH, JOWETT, PAULY, ABDERHALDEN and BERGELL, FRIEDMANN and STOLZ.[1] v. FÜRTH calls it *suprarenin*, ABEL *epinephrin*, and TAKAMINE *adrenalin*. This last name seems to be the most generally accepted one.

Adrenalin (suprarenin epinephrin), $C_9H_{13}NO_3$,



The constitution of adrenalin has been essentially proven by FRIEDMANN,[2] and he has shown the correctness of the above formula, which was given by PAULY. The synthesis of adrenalin, which was first performed by STOLZ,[3] is also in accordance with this formula. By the action of methylamine upon chloracetopyrocatechin we obtain methylaminoaceto-pyrocatechin:

$$C_6H_3(OH)_2.COCH_2Cl + NH_2CH_3 = C_6H_3(OH)_2.COCH_2.NHCH_3.HCl,$$

which yields adrenalin on reduction.

The synthetically prepared adrenalin is optically inactive *d-l*-adrenalin, while that from the adrenals is optically active *l*-adrenalin. FLÄCHER has recently divided the racemic adrenalin into the two optically active components, and the identity of the so-obtained synthetical adrenalin with the natural has been shown by ABDERHALDEN and FR. MÜLLER.[4] These last investigators also found that the *l*-adrenalin had at least 15 times as strong an action upon the blood-pressure as the *d*-adrenalin, and later ABDERHALDEN with THIES and SLAVU found that the *l*-adrenalin had also in other respects a much stronger action than *d*-adrenalin.

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[1] The literature on this subject may be found in v. Fürth, Zeitschr. f. physiol. Chem., **23**, **26**, **29** and Wien. Sitzungsber. Math. Nat. Kl., **112**, 1903. See also Abel, Zeitschr. f. physiol. Chem., **28**; Amer. Journ. of Physiol., 1899, and The Johns Hopkins Hospital Bull., No. **76** (1897), **90** and **91** (1898), **120** and **128** (1901), **131** and **132** (1902); Ber. d. d. chem. Gesellsch., **36**; Abel and Taveau, Journ. of Biol. Chem., **1**, and Friedmann, Hofmeister's Beiträge, **6** and **8**.

[2] Hofmeister's Beiträge, **8**.

[3] Ber. d. d. chem. Gesellsch., **37**.

[4] Flächer, Zeitschr. f. physiol. Chem., **58**; Abderhalden and Franz Müller, *ibid.*, **58**; with Thies, *ibid.*, **59**; with Slavu, *ibid.*, **59**.

Adrenalin crystallizes in masses of needles or rhombic leaves. It is soluble in water, and can be precipitated from its solution by ammonia as a crystalline substance. Its aqueous solution containing hydrochloric acid is levorotatory: $(\alpha)_D = -50.72°$ (ABDERHALDEN and GUGGENHEIM [1]). On heating adrenalin it turns yellowish brown at about 205° and decomposes at about 218° C. Its solution turns emerald green with ferric chloride in acid solution and carmine red in alkaline solution. Adrenalin reduces FEHLING'S solution and ammoniacal silver solution.

As above stated, it has been considered for some time that the color of the skin in ADDISON'S disease was connected with the adrenals or their chromogen. We know nothing positive in regard to this relation, but it is nevertheless of interest that pigments and finally melanins or at least dark-brown substances can be produced from adrenalin by the action of enzymes. NEUBERG has brought about such melanin formation by the extract from the metastases of a melanoma of the adrenals and also with the extract of the ink-sac of the sepia, and ABDERHALDEN and GUGGENHEIM [2] with tyrosinase. This would indicate a close relation between adrenalin and tyrosin, which also gives melanin with the sepia enzyme, and indeed tyrosin has been considered as the probable mother substance of adrenalin (HALLE [3]). From the above-mentioned experiments as well as from the investigations of ABELOUS, SOULIÉ and TOUJAN [4] on the formation of adrenalin in the adrenals under the coaction of autolytic products of other organs or organ extracts, no positive conclusions can be drawn.

Besides the action of producing a rise in the blood-pressure, adrenalin is also of special interest because, as first shown by BLUM,[5] it also has a glycosuric action. We will discuss the question of adrenalin glycosuria and the relation which seems to exist between the internal secretions of the thyroids, the adrenals and the pancreas, when we treat of the formation of sugar and pancreas diabetes.

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[1] Zeitschr. f. physiol. Chem., 57.

[2] Neuberg, Bioch. Zeitschr., 8; Abderhalden and Guggenheim, Zeitschr. f. physiol. Chem., 57.

[3] Halle, Hofmeister's Beiträge, 8.

[4] Compt. rend. soc. biol., 58, 59, 60.

[5] Deutsch. Arch. f. klin. Med., 91, and Pflüger's Arch., 90.

CHAPTER VIII.

THE LIVER.

The liver, which is the largest gland of the body, stands in close relation to the glands mentioned in Chapter VII. The importance of this organ for the assimilation of the food-stuffs and for the physiological composition of the blood is evident from the fact that the blood coming from the digestive tract, laden with absorbed bodies, must circulate through the liver before it is driven by the heart through the different organs and tissues. We are not clear as to what extent an assimilation of the absorbed digestion products of the proteins brought to the liver by the portal blood takes place in this organ; for the carbohydrates it has been proven that glycogen is formed from the dextrose. By this glycogen formation the liver becomes the real reserve-organ for the carbohydrates: it is also a storage-organ for fat, and perhaps also for proteins.[1]

The formation of glycogen from dextrose is a synthesis, and the occurrence of other syntheses in the liver has been repeatedly shown by special observations. For example, in the liver certain ammonia combinations are converted into urea or uric acid (in birds) (see Chapter XV), while certain products of putrefaction in the intestine, such as phenols, may be converted by synthesis into ethereal sulphuric acids by the liver (Pflüger and Kochs, Embden and Glaessner), probably also converted into conjugated glucuronic acids (Embden[2]). The liver has also the property of removing and retaining heterogeneous bodies from the blood, and this is true not only of metallic salts, which are often removed by this organ, but also, as Schiff, Heger, and others, but especially Roger, have shown, the alkaloids are retained, and are probably also partially decomposed in the liver. Toxines are also withheld by the liver, and hence this organ has a protective action against poisons.[3]

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[1] See Seitz, Pflüger's Arch., **111**, and Asher and Boehm, Zeitschr. f. Biol., **51**.

[2] Pflüger and Kochs, Pflüger's Arch., **20** and **23**; Embden and Glaessner, Hofmeister's Beiträge, **1**; Embden, *ibid.*, **2**.

[3] Roger, Action du foie sur les poisons (Paris, 1887), which also contains the older literature; Bouchard, Leçons sur les autointoxications dans les maladies (Paris, 1887); and E. Kotliar in Arch. des sciences biologiques de St. Pétersbourg, **2**. See also de

Even though the liver is of assimilatory importance and purifies the blood coming from the digestive tract, it is at the same time a secretory organ which eliminates a specific secretion, the bile, in the production of which the red blood-corpuscles are destroyed, or at least one of their constituents, the hæmoglobin. It is generally admitted that the liver acts contrariwise during fœtal life, at that time forming the red blood-corpuscles.

There is no doubt that the chemical operations going on in this organ are manifold and must be of the greatest importance to the organism. Our knowledge on this subject has been essentially advanced by recent investigations, but nevertheless it must be admitted that we know little of the character and extent of these changes. Among the products of these chemical processes there are two which are especially important and must be treated in this chapter, namely, the glycogen and the bile. Before the study of these products is taken up, a short discussion of the constituents and the chemical composition of the liver is necessary.

The reaction of the liver-cells is alkaline toward litmus during life, but becomes acid after death, due to a formation of lactic acid, chiefly fermentation lactic acid and other organic acids (Morishima, Magnus-Levy [1]). A coagulation of the protoplasmic proteins in the cells probably takes place. A positive difference between the proteins of the dead and the living, non-coagulated protoplasm has not been observed.

The *proteins* of the liver were first carefully investigated by Plósz. He found in the watery extract of the liver an *albuminous substance* which coagulates at 45° C., (globulin, Halliburton) also a *globulin* which coagulates at 75° C., a *nucleoalbumin* which coagulates at 70° C., and lastly a protein body which is closely related to the *coagulated albumins* and which is insoluble in dilute acids or alkalies at the ordinary temperature, but dissolves on the application of heat, being converted into an albuminate. Halliburton [2] found two globulins in the liver-cells, one of which coagulates at 68–70° C., and the other at 45–50° C. He also found, besides traces of albumin, a nucleoprotein which possessed 1.45 per cent phosphorus and a coagulation-point of 60° C. Pohl has obtained an "organ plasma" by extracting the finely divided liver which had previously been entirely freed from blood by washing with 8 p. m. NaCl solution, in which he was able to detect a globulin having a low coagulation temperature. The very variable phosphorus content (0.28–1.3 per cent) of this globulin as well as the insolubility of the pre-

Vamossy, Centralbl. f. Physiol., **18**, and Rothberger, Wien. klin. Wochenschr., 1905, Rothberger and Winterberg, Biochem. Centralbl., **4**.

[1] Morishima, Arch. f. exp. Path. u. Pharm., **43**; Magnus-Levy, Hofmeister's Beiträge, **2**.

[2] Plósz, Pflüger's Arch., **7**; Halliburton, Journ. of Physiol., **13**, Suppl. 1892.

cipitates produced by little acid, in an excess of acid, and in neutral salts seem to indicate that we here have a mixture which consists chiefly of nucleoproteins and not of globulins. The nearly complete digestibility with pepsin-hydrochloric acid does not controvert this assumption, because, as is known, nucleoproteins may on digestion yield no residue (see Chapter III). Nor can we be positive concerning the nature of the liver-globulin found by DASTRE,[1] having a coagulation temperature of 56°. The proteins extractable from the liver without modification must be thoroughly investigated.

Besides the above-mentioned proteins, which are very soluble, the liver-cells contain large quantities of difficultly soluble protein bodies (see PLÓSZ). The liver also contains, as first shown by ST. ZALESKI and later substantiated by several other investigators, ferruginous proteins of different kinds.[2] The chief portion of the protein substances in the liver seems in fact to consist of ferruginous nucleoproteins. On boiling the liver with water, such a nucleoprotein or perhaps several are split, and a solution is obtained containing a nucleic-acid-rich nucleoprotein or a mixture of these which are precipitable by acids. This protein or protein mixture, which has been called *ferratin* by SCHMIEDEBERG,[3] has been studied by WOHLGEMUTH.[4] The quantity of phosphorus was 3.06 per cent. As cleavage products on hydrolysis he found *l*-xylose, the four nuclein bases, and also arginine, lysine (and histidine?), tyrosine, leucine, glycocoll, alanine, α-proline, glutamic acid, aspartic acid, phenylalanine, oxyaminosuberic acid, and oxydiaminosebacic acid (see Chapter III). The *l*-xylose depends, no doubt, at least in part, upon the guanylic acid isolated from the liver, by LEVENE and MANDEL,[5] and the finding of adenine among the cleavage products also indicates the presence of a thymonucleic acid. There does not seem to be any doubt that the ferratin, as above stated, is a mixture, and the correctness of this assumption is shown by the recent investigations of SCHAFFIDI and SALKOWSKI.[6]

The yellow or brown pigment of the liver has been little studied. DASTRE and FLORESCO [7] differentiate, in vertebrates and certain invertebrates, between a

[1] Pohl, Hofmeister's Beiträge, **7**; Dastre, Compt. rend. soc. biolog., **58**.

[2] St. Zaleski, Zeitschr. f. physiol. Chem., **10**, 486; Woltering, *ibid.*, **21**; Spitzer, Pflüger's Arch., **67**.

[3] Arch. f. exp. Path. u. Pharm., **33**; see also Vay, Zeitschr. f. physiol. Chem., **20**.

[4] Wohlgemuth, Zeitschr. f. physiol. Chem., **37**, **42**, and **44**, and Ber. d. d. chem. Gesellsch., **37**. See on liver nucleoproteins also Salkowski, Berl. klin. Wochenschr., 1895; Hammarsten, Zeitschr. f. physiol. Chem., **19**; Blumenthal, Zeitschr. f. klin. Med., **34**.

[5] Bioch. Zeitschr., **10**.

[6] Schaffidi, Zeitschr. f. physiol. Chem., **58**; Salkowski, *ibid.*, **58**.

[7] Arch. de Physiol. (5), **10**.

ferruginous pigment soluble in water, *ferrine*, and a pigment soluble in chloroform and insoluble in water, *chlorochrome*. They have not isolated these pigments in a pure condition. In certain invertebrates chlorophyll originating from the food also occurs in the liver.

The *fat* of the liver occurs partly as very small globules and partly (especially in nursing children and suckling animals, as also after food rich in fat) as rather large fat-drops. The occurrence of a fatty infiltration, i.e., a transportation of fat to the liver, may not only be produced by an excess of fat in the food (Noël-Paton), but also by a migration from other parts of the body under abnormal conditions, such as poisoning with phosphorus, phlorhizin, and certain other bodies (Leo, Lebedeff, Rosenfeld, and others [1]). The fatty infiltration occurring in poisoning, and which is accompanied with degenerative changes in the cells, may cause a diminution in the amount of protein and a rise in the water content. If the amount of fat in the liver is increased by an infiltration, the water decreases correspondingly, while the quantity of the other solids remains little changed. Changes of a kind may occur, so that, because of the antipathy (Rosenfeld, Bottazzi)[2] existing between glycogen and fat, a liver rich in fat is habitually poor in glycogen. The reverse occurs after feeding with carbohydrate-rich food, namely, the liver is rich in glycogen and poor in fat.

The composition of the liver-fat seems to vary not only in different animals, but is variable with differing conditions. Thus Noël-Paton found that the liver-fat in man and several animals was poorer in oleic acid and had a correspondingly higher melting-point than the fat from the subcutaneous connective tissue, while Rosenfeld [3] observed the opposite condition on feeding dogs with mutton-fat.

The deductions as to the quantity of glycerides with stronger unsaturated acids than oleic acid in liver fat are to be accepted with caution, as these acids originate from contaminated phosphatides.

Phosphatides, which were formerly designated lecithin, and whose quantity is generally calculated as such, also belong to the normal constituents of the liver. The quantity (as lecithin) amounts to over 23.5 p. m. according to Noël-Paton.[4] In starvation the lecithin, according to Noël-

[1] Noël-Paton, Journ. of Physiol., **19**; Leo, Zeitschr. f. physiol. Chem., **9**; Lebedeff Pflüger's Arch., **31**; Athanasiu, Pflüger's Arch., **74**; Taylor, Journ. of Exp. Med., **4**; Kraus u. Sommer, Hofmeister's Beiträge, **2**; Rosenfeld, Zeitschr. f. klin. Med., **36**. See also Rosenfeld, Ergebnisse der Physiologie, **1**, Abt. **1**, and Berl. klin. Wochenschr., 1904; Schwalbe, Centralbl. f. Physiol., **18**, p. 319.

[2] Arch. Ital. d. Biol., 48 (1908), cited in Bioch. Centralbl., **7**, p. 833.

[3] Cited by Lummert, Pflüger's Arch., **71**. In regard to the liver-fat of children, see Thiemich, Zeitschr. f. physiol. Chem., **26**.

[4] l. c. See also Heffter, Arch. f. exp. Path. u. Pharm., **28**.

Paton, forms the greater part of the ethereal extract, while with food rich in fat, on the contrary, it forms the smaller part. The phosphatides are undoubtedly of various kinds, but they have not been closely studied. Among others, we have *lecithin* and the so-called *jecorin*.[1] *Cholesterin* is also a constituent of the liver, although only in small quantities.

Jecorin was first found by Drechsel in the liver of horses, and also in the liver of a dolphin, and later by Baldi in the liver and spleen of other animals, in the muscles and blood of the horse, and in the human brain. It contains sulphur and phosphorus, but its constitution is not positively known. Jecorin dissolves in ether, but is precipitated from this solution by alcohol. It reduces copper oxide, and gives a wine-red coloration with an ammoniacal silver-solution. On boiling with alkali and then cooling it solidifies to a gelatinous mass. Manasse has detected dextrose as osazone in the carbohydrate complex of jecorin.

The statement by Bing that jecorin is a combination of lecithin and dextrose does not follow from the analyses of jecorin thus far known. Jecorin contains sulphur, even as much as 2.75 per cent, and further the relation of P:N in lecithin is 1:1, while in jecorin it is quite different, 1:2 to 1:6. According to the recent investigations of Baskoff the liver jecorin, prepared according to Drechsel's suggestion, and when it is so pure that it is completely soluble in ether, and quantitatively precipitated by alcohol, from this solution is a rather constant compound at least in regard to the N, P and dextrose content. Baskoff found as average 2.55 per cent N, 2.87 per cent P, and about 14 per cent dextrose. The relation P:N was nearly 1:2 and therefore jecorin is correspondingly a diamidomonophosphatide.

The variable composition and divergent properties of the jecorin isolated and analyzed by various investigators [2] depends, according to Baskoff, upon imperfect purification. His investigations do not give any explanation for the quantity of sulphur and it is very probable that jecorin is only a mixture of several bodies, among which a sulphurized and a phosphorized substance occurs.

Another phosphatide, which does not reduce directly or after boiling with acid, has been called *heparphosphatide* by Baskoff. In certain regards this body is similar to cuorin, and the relation P:N = 1.45:1, although it was not pure.

Among the *extractive substances* besides *glycogen*, which will be treated later, rather large quantities of the *purine bases* occur. Kossel [3] found in 1000 parts of the dried substance 1.97 p. m. *guanine*, 1.34 p. m. *hypoxanthine*, and 1.21 p. m. *xanthine*. *Adenine* is also contained in the liver. In addition there are found *urea* and *uric acid* (especially in birds), and indeed in larger quantities than in the blood, *paralactic acid*, *leucine*, and *cystine*. In pathological cases *inosite* and *amino-acids* have been detected. The occurrence of *bile-coloring matters* in the liver-

[1] See Baskoff, Zeitschr. f. physiol. Chem., 57.

[2] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1886, p. 44, and Zeitsch. f. Biologie, 33; Baldi, Arch. f. (Anat. u.) Physiol., 1887, Suppl., 100; Manasse, Zeitschr. f. physiol. Chem., 20; Bing, Centralbl. f. Physiol., 12, and Skand. Arch. f. Physiol., 9; Meinertz, Zeitschr. f. physiol. Chem., 46; Siegfried and Mark, *ibid.*; Paul Mayer, Bioch. Zeitschr., 1, and Baskoff, Zeitschr. f. physiol. Chem., 57.

[3] Zeitschr. f. physiol. Chem., 8.

cell under normal conditions is doubtful; but in retention of the bile the cells may absorb the coloring-matter and become colored thereby.

A large number of enzymes are found in the liver, such as *catalases*, *oxidases*, the *glycolytic enzymes*, which will be spoken of later, the enzymes taking part in the *formation of uric acid* and *destruction of uric acid* (Chapter XV), the *arginase*, which forms *urea*, and the *diastase* acting upon glycogen, the ester-splitting *lipases* and the *proteolytic enzymes*.[1]

The proteolytic enzymes of the liver are of special interest, especially in regard to the study of the autolysis of this organ. The processes in the liver in phosphorus poisoning and in acute yellow atrophy of the liver are considered as an intravitally increased autolysis. In these cases a softening of the organ takes place, and proteoses, mono- and diamino-acids, and other bodies are produced, which may also be found in the urine, and although they may not all be derived from the liver (Neuberg and Richter), they are at least in part derived from this organ. Wakeman has found in phosphorus poisoning that not only is the quantity of nitrogen markedly diminished in the liver (of dogs), but also that the quantity of nitrogen of the hexone bases is diminished, and that the part of the protein molecule richer in nitrogen is first removed and eliminated under these conditions. A similar condition has been observed by Wells in the idiophatic, acute yellow atrophy of the liver. In consideration of the variable results for the diamino-nitrogen even under normal conditions (Glikin and A. Loewy[2]), it is desirable that a greater number of observations be made on this subject. The increased consumption of glycogen under the above-mentioned pathological conditions may also be considered as an increased autolysis, while the claim of certain observers that fat is formed in the autolysis of the liver is according to Saxl[3] to be considered only as a more pronounced appearance of the fat previously occurring in the organ.

Besides the above-mentioned organic constituents in the liver we must mention the *glucothionic acid* found by Mandel and Levene, whose chemical individuality is doubted, as well as the nitrogenous carbohydrate found in the liver by Seegen and Neimann which also requires further investigation, and whose occurrence in the liver could not be substantiated by Türkel.[4]

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[1] Granström, Hofmeister's Beiträge, **11**, claims to have found in the liver an enzyme which he calls *glyoxylase*, which destroys glyoxylic acid.

[2] Neuberg and Richter, Deutsch. med. Wochenschr., 1904; Wakeman, Zeitschr. f. physiol. Chem., **44**; Wells, Journ. of Exper. Med., **9**; Glikin and Loewy, Bioch. Zeitschr., **10**.

[3] Hofmeister's Beiträge, **10**.

[4] Mandel and Levene, Zeitschr. f. physiol. Chem., **45**; Seegen, Centralbl. f. Physiol.,

The *mineral bodies* of the liver consist of phosphoric acid, potassium, sodium, alkaline earths, and chlorine. The potassium is in excess of the sodium. Iron is a regular constituent of the liver, but it occurs in very variable amounts. BUNGE found 0.01–0.355 p. m. iron in the blood-free liver of young cats and dogs. This was calculated on the liver substance freshly washed with a 1 per cent NaCl solution. Calculated on 10 kilos bodily weight, the iron in the liver amounted to 3.4–80.1 mg. Recent determinations of the quantity of iron in the liver of the rabbit, dog, hedge-hog, pig, and man have been made by GUILLEMONAT and LAPICQUE, and in rabbits by SCAFFIDI. The variation was great in human beings. In men the quantity of iron in the blood-free liver (blood-pigment subtracted in the calculation) was regularly more, and in women less, than 0.20 p. m. (calculated on the fresh moist organ). Above 0.5 p. m. is considered as pathological. According to BIELFELD,[1] who also finds a greater iron content in men, this difference appears only after the first 20–25 years. At this age (20–25 years) the iron content is smallest.

The quantity of iron in the liver can be increased by drugs containing iron, as also by inorganic iron salts, and the largest deposition of iron was observed by NOVI[2] after the hypodermic injection of iron. The quantity of iron may also be increased by an abundant destruction of red blood-corpuscles, which will result from the injection of dissolved hæmoglobin, in which process the iron combinations derived from the blood-pigments in other organs, such as the spleen and marrow, also seem to take part.[3] A destruction of blood-pigments, with a splitting off of compounds rich in iron, seems to take place in the liver in the formation of the bile-pigments. Even in invertebrates, which have no hæmoglobin, the so-called liver is rich in iron, from which DASTRE and FLORESCO[4] conclude that the quantity of iron in the liver of invertebrates is entirely independent of the decomposition of the blood-pigment, and in vertebrates it is in part so. According to these authors the liver has, on account of the quantity of iron, a specially important oxidizing function, which they call the "*fonction martiale*" of the liver.

The richness in iron of the liver of new-born animals is of special interest—a condition which was shown by the analyses of ST. ZALESKI,

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12 and **13**, with Neimann, Wiener Sitzungsber. Math. Klasse, **112**; Türkel, Hofmeister's Beiträge, **9**.

[1] Bunge, Zeitschr. f. physiol. Chem., **17**, 78; Guillemonat and Lapicque, Compt. rend. de soc. biol., 48, and Arch. de Physiol. (5), 8; Bielfeld, Hofmeister's Beiträge, **2**; see also Schmey, Zeitschr. f. physiol. Chem., **39**; Scaffidi, *ibid.*, **54**.

[2] See Centralbl. d. Physiol., **16**, 393.

[3] See Lapicque, Compt. rend., **124**, and Schurig, Arch. f. exp. Path. u. Pharm., **41**.

[4] Arch. de Physiol. (5), **10**.

but was especially studied by KRÜGER and MEYER. In oxen and cows they found 0.246–0.276 p. m. iron (calculated on the dry substance), and in the cow-fœtus about ten times as much. The liver-cells of a calf a week old contain about seven times as much iron as the adult animal; the quantity decreases in the first four weeks of life, when it reaches about the same amount as in the adult. LAPICQUE [1] also found that in rabbits the quantity of iron in the liver steadily diminishes from the eighth day to three months after birth, namely, from 10 to 0.4 p. m., calculated on the dry substance. "The fœtal liver-cells bring an abundance of iron in the world to be used up, within a certain time, for a purpose not well known." A part of the iron exists as phosphate, but the greater part is in combination in the ferruginous protein bodies (ST. ZALESKI).

The quantity of calcium oxide in the fresh, moist liver of the horse, ox, and pig, according to TOYONAGA, amounts to 0.148–0.193 p. m., or about the same as in the human liver. The amount of magnesium oxide was remarkably high, namely, 0.168, 0.198, and 0.158 p. m., in the livers of the horse, ox, and pig respectively. KRÜGER [2] found the quantity of calcium in the livers of adult cattle and of calves to be respectively 0.71 p. m. and 1.23 p. m. of the dried substance. In the fœtus of the cow it is lower than in calves. During pregnancy the iron and calcium in the fœtus are antagonistic; that is, an increase in the quantity of calcium in the liver causes a diminution in the iron, and an increase in the iron causes a decrease in the calcium. Copper seems to be a physiological constituent, and occurs to a considerable extent in cephalopods (HENZE [3]). Foreign metals, such as lead, zinc, arsenic, and others (also iron), are easily taken up and combined by the liver (SLOWTZOFF, v. ZEYNEK, and others [4]).

v. BIBRA [5] found in the liver of a young man who had suddenly died 762 p. m. water and 238 p. m. solids, consisting of 25 p. m. fat, 152 p. m. protein, gelatin-forming and insoluble substances, and 61 p. m. extractive substances.

PROFITLICH [6] found 68.2–75.17 per cent water in the dog liver and 70.76–72.86 per cent in the ox liver. The relation N:C in the fat and glycogen-free dried substance was 1:3.21 in dogs and 1:3.13 in oxen or about the same as in flesh (see Chapter XI).

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[1] St. Zaleski, l. c.; Krüger and collaborators, Zeitschr. f. Biologie, **27**; Lapicque, Maly's Jahresber., **20**.

[2] Zeitschr. f. Biologie, **31**; Toyonaga, Bull. of the College of Agriculture, Tokio, **6**.

[3] Zeitschr. f. physiol. Chem., **33**.

[4] Slowtzoff, Hofmeister's Beiträge, **1**; v. Zeynek, see Centralbl. f. Physiol., **15**.

[5] See v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., p. 711.

[6] Pflüger's Arch., **119**.

The quantitative composition of the liver may show great variation, depending upon the kind and amount of the food supplied. The amount of carbohydrate (glycogen) and fat may vary considerably, which is due to the fact that the liver is a storage-organ for these bodies, especially for the glycogen.

Based upon special experiments, SEITZ [1] claims that the liver is a storehouse for protein also. In experiments on hens and ducks which had previously been starved, he found that the liver took up abundant protein on feeding meat, and that its weight as compared with the weight after starvation was doubled or quadrupled. As it is characteristic of storage or reserve bodies that their amount in the storage-organs on feeding with such bodies strongly increases in percentage, it is remarkable in SEITZ's feeding experiments that the percentage of protein in the liver did not increase, but rather diminished slightly. In this case we did not have a higher percentage of protein, but an increase in the weight of the total cell mass of the organ, probably brought about by increased work of the liver due to the protein feeding. It is also difficult to decide as to how far in these experiments we are dealing with an increase in the number or the size of the liver-cells or with a deposition of reserve protein in the same sense as of glycogen or excessive fat.

There is a unanimous belief that the liver is an especially important storage-organ for glycogen.

Glycogen and its Formation.

Glycogen was first discovered by BERNARD. It is a carbohydrate closely related to the starches or dextrins, with the general formula $m(C_6H_{10}O_5)$. Its molecular weight is unknown, but seems to be very large (GATIN-GRUZEWSKA and v. KNAFFL-LENZ [2]). The largest quantities are found in the liver of adult animals, and smaller quantities in the muscles (BERNARD, NASSE). It is found in very small quantities in nearly all tissues of the animal body. Its occurrence in lymphoid cells, blood, and pus has been mentioned in a previous chapter, and it seems to be a regular constituent of all cells capable of development. Glycogen was first shown to exist in embryonic tissues by BERNARD and KÜHNE, (see also MENDEL and LEAVENWORTH [3]), and it seems on the whole to be a constituent of tissues in which a rapid cell formation and cell development are taking place. It is also present in rapidly forming pathological tumors (HOPPE-SEYLER). Some animals, as certain mussels

[1] Pflüger's Arch., **111**.

[2] Gatin-Gruzewska, Pflüger's Arch., **103**; v. Knaffl-Lenz, Zeitschr. f. physiol. Chem., **46**.

[3] Amer. Journ. of Physiol., **20**.

(Bizio), Tænia and Ascaridæ (Weinland [1]), are very rich in glycogen. Glycogen also occurs in the vegetable kingdom, especially in many fungi.

The quantity of glycogen in the liver, as also in the muscles, depends essentially upon the food. In starvation it disappears almost completely after a short time, but more rapidly in small than in large animals, and it disappears earlier from the liver than from the muscles. As shown by C. Voit, Külz and especially by Pflüger,[2] it never entirely disappears in starvation, as a reformation of glycogen always takes place. After partaking of food, especially such as is rich in carbohydrates, the liver becomes rich again in glycogen, the greatest increment occurring 14 to 16 hours after eating (Külz). The quantity of liver-glycogen may amount to 120–160 p. m. after partaking of large quantities of carbohydrates, and in dogs which had been especially fed for glycogen Schöndorff and Gatin-Gruzewska found still higher results, even more than 180 p. m. Ordinarily it is considerably less, namely, 12–30 to 40 p. m. The highest amount of glycogen in the liver thus far observed was 201.6 p. m., found by Mangold [3] in the frog. The shark, whose liver is very rich in fat, even though well nourished, only has comparatively low values for the glycogen in the liver, 9.3–23.8 p. m. (Bottazzi[4]). According to Cremer the quantity of glycogen in plants (yeast-cells) is, as in animals, dependent upon the food. He finds that the yeast-cells contain glycogen, which disappears from the cells in the auto-fermentation of the yeast, but reappears on the introduction of the cells into a sugar solution.

The quantity of glycogen of the liver (and also of the muscles) is also dependent upon rest and activity, because during rest, as in hibernation, it increases, and during work it diminishes. Külz has shown that by hard work the quantity of glycogen in the liver (of dogs) is reduced to a minimum in a few hours. The muscle-glycogen does not diminish to the same extent as the liver-glycogen. Külz, Zuntz and Vogelius, Frentzel, and others have been able to render rabbits and frogs nearly glycogen-free by suitable strychnine poisoning. The same result is produced by starvation followed by hard work. According to Gatin-Gruzewska, [5] the liver and muscles in rabbits can be made glycogen-

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[1] Zeitschr. f. Biologie, **41**. The extensive literature on glycogen may be found in E. Pflüger, Glykogen, 2. Aufl., Bonn, 1905; and in Cremer, "Physiol. des Glykogens," in Ergebnisse der Physiologie, **1**, Abt. 1. In the following pages we shall refer to these works.

[2] Pflüger's Arch., **119**, which contains the literature.

[3] Pflüger's Arch., **121**.

[4] Arch. Ital. d. Biol., 48; cited in Bioch. Centralbl., **7**, 833.

[5] Compt. rend., **142**.

free after 36–40 hours by first starving one day and then injecting adrenalin.

Glycogen forms an amorphous, white, tasteless, and inodorous powder. When perfectly pure, and by proper alcohol precipitation, it can be obtained as rods or prisms which look like crystals (GATIN-GRUZEWSKA). It gives an opalescent solution with water which, when allowed to evaporate on the water-bath, forms a pellicle over the surface that disappears again on cooling. It is undecided whether we here have a true solution or not. Like other colloids, glycogen in water under the influence of the electric current migrates to the anode, on which it collects (GATIN-GRUZEWSKA). Its aqueous solution is dextrorotatory, and HUPPERT found it to be $(\alpha)_D = +196.63°$. GATIN-GRUZEWSKA has recently obtained the same result by using a perfectly pure solution of glycogen.[1] A solution of glycogen, especially on the addition of NaCl, is colored wine-red by iodine. It may hold cupric hydroxide in solution in alkaline liquids, but does not reduce it. A solution of glycogen in water is not precipitated by potassium-mercuric iodide and hydrochloric acid, but is precipitated by alcohol (on the addition of NaCl when necessary), or ammoniacal basic lead acetate. An aqueous solution of glycogen made alkaline with caustic potash (15 per cent KOH) is completely precipitated by an equal volume of 96-per cent alcohol. Tannic acid also precipitates glycogen. It gives a white granular precipitate of benzoyl-glycogen with benzoyl chloride and caustic soda. Glycogen is completely precipitated by saturating its solution at ordinary temperatures with magnesium or ammonium sulphate. It is not precipitated by sodium chloride, or by half saturation with ammonium sulphate (NASSE, NEUMEISTER, HALLIBURTON, YOUNG[2]). On boiling with dilute caustic potash (1–2 per cent) the glycogen may be more or less changed, especially if it has been previously exposed to the action of acid or to BRÜCKE'S reagent (see below) (PFLÜGER). On boiling with stronger caustic potash (even of 36-per cent) it is not injured (PFLÜGER). By diastatic enzymes glycogen is converted into maltose or dextrose, depending upon the nature of the enzyme. It is transformed into dextrose by dilute mineral acids. According to TEBB[3] various dextrins appear as intermediary steps in the saccharification of glycogen, depending on whether the hydrolysis is caused by mineral acids or enzymes. The question whether the glycogen from various animals and different organs is the same in this regard has not been sufficiently investigated. Nor has it been decided whether

[1] Bottazzi and d'Errico (Pflüger's Arch., 115) have investigated the viscosity, the electrical conductivity and the freezing-point of glycogen solutions at different concentrations.

[2] Young, Journ. of Physiol., 22, citing the other investigators.

[3] Journ. of Physiol., 22.

all the glycogen in the liver occurs as such or whether it is in part combined with protein (PFLÜGER-NERKING). The investigations of LOESCHCKE [1] have shown that we have no positive reasons for this assumption.

The preparation of pure glycogen (most easily from the liver) is generally performed by the method suggested by BRÜCKE, of which the main points are the following: Immediately after the death of the animal the liver is thrown into boiling water, then finely divided and boiled several times with fresh water. The filtered extract is now sufficiently concentrated, allowed to cool, and the proteins removed by alternately adding potassium-mercuric iodide and hydrochloric acid. The glycogen is precipitated from the filtered liquid by the addition of alcohol until the liquid contains 60 vols. per cent. By repeating this and precipitating the glycogen several times from its alkaline and acetic-acid solution it is purified on the filter by washing first with 60-per cent and then with 95-per cent alcohol, then treating with ether, and drying over sulphuric acid. It is always contaminated with mineral substances. To be able to extract the glycogen from the liver or, especially, from muscles and other tissues completely, which is essential in a quantitative estimation, these parts must first be warmed for two hours with strong caustic potash (30-per cent) on the water-bath. As the glycogen changes in this purification, as suggested by BRÜCKE, it is better, for quantitative determinations of glycogen, to precipitate it directly from the alkaline solution by alcohol (PFLÜGER [2]).

The quantitative estimation is best performed according to PFLÜGER'S method, which is as follows: The finely divided organ is heated on the water-bath for 2–3 hours in the presence of 30-per cent KOH; after diluting with water and filtering, the glycogen is precipitated with alcohol, and the redissolved glycogen estimated in part by the polariscope and in part as sugar after inversion. One part by weight of sugar equals 0.927 part glycogen. As in the estimation the prescribed directions must be exactly followed, we must refer to the original work of PFLÜGER for the details of the method. Other methods of estimating glycogen, such as those of BRÜCKE-KÜLZ, PAVY, and AUSTIN, are described in PFLÜGER'S Archiv. 96. Also compare the recent works of PFLÜGER [3] and BANG.[4]

Numerous investigators have endeavored to determine the origin of glycogen in the animal body. It is positively established by the unanimous observations of many investigators [5] that the varieties of *sugars* and their anhydrides, *dextrins* and *starches*, have the property of

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[1] Pflüger's Arch., **102**.

[2] See also the method suggested by Gautier, Compt. rend., **129**.

[3] Pflüger's Arch., **103**, **104**, **121**.

[4] Hammarsten's Festschr., 1906.

[5] In reference to the literature on this subject, see E. Külz, Pflüger's Arch., **24**, and Ludwig, Festschrift, 1891; also the cited works of Pflüger and Cremer, foot-note 1, p. 369.

increasing the quantity of glycogen in the body. The action of inulin seems to be somewhat uncertain.[1] The statements are questioned in regard to the action of the pentoses. CREMER found that in rabbits and hens various pentoses, such as rhamnose, xylose, and arabinose, have a positive influence on the glycogen formation, and SALKOWSKI obtained the same result on feeding *l*-arabinose. FRENTZEL, on the contrary, found no glycogen formation on feeding xylose to a rabbit which had previously been made glycogen-free by strychnine poisoning, and NEUBERG and WOHLGEMUTH [2] obtained similar negative results on feeding rabbits with *d*- and *r*-arabinose. In general we can for the present accept the view that the pentoses are not direct glycogen formers.

The hexoses, and the carbohydrates derived therefrom, do not all possess the ability of forming or accumulating glycogen to the same extent. Thus C. VOIT [3] and his pupils have shown that dextrose has a more powerful action than cane-sugar, while milk-sugar is less active (in rabbits and hens) than dextrose, levulose, cane-sugar, or maltose. The following substances when introduced into the body also increase the quantity of glycogen in the liver: *Glycerin, gelatin, arbutin,* and likewise, according to the investigations of KÜLZ, *erythrite, quercite, dulcite, mannite, inosite, ethylene* and *propylene glycol, glucuronic anhydride, saccharic acid, mucic acid, sodium tartrate, saccharin, isosaccharin,* and *urea. Ammonium carbonate, glycocoll,* and *asparagine* may similarly, according to RÖHMANN, cause an increase in the amount of glycogen in the liver. NEBELTHAU finds that other ammonium salts and some of the amides, as well as certain *narcotics, hypnotics,* and *antipyretics,* produce an increase in the glycogen of the liver. This action of the antipyretics (especially antipyrine) had been shown by LÉPINE and PORTERET.[4]

PFLÜGER has conclusively shown that we have no positive proof as to the action of these various bodies as glycogen-formers. That glycerin may in a positive sense influence the amount of glycogen in the liver is not to be doubted from the experiments of WEISS and LUCHSINGER on glycogen formation, which will be mentioned in connection with the experiments on the relation of glycerin to the formation of sugar.

The fats, according to BOUCHARD and DESGREZ, increase the glycogen content of the muscles but not of the liver, while COUVREUR [5] believes that the glycogen is increased at the expense of the fat in the silkworm larva

[1] See Miura, Zeitschr. f. Biologie, **32**, and Nakaseko, Amer. Journ. of Physiol., **4**.

[2] Salkowski, Zeitschr. f. physiol. Chem., **32**; Neuberg and Wohlgemuth, *ibid.*, **35**. See also Pflüger, l. c., and Cremer, l. c.

[3] Zeitschr. f. Biologie, **28**.

[4] Röhmann, Pflüger's Arch., **39**; Nebelthau, Zeitschr. f. Biologie, **28**; Lépine and Porteret, Compt. rend., **107**.

[5] Bouchard et Desgrez, Compt. rend., **130**; Couvreur, Compt. rend. de soc. biol., **47**.

as it changes into a chrysalis. In general it is believed that fat does not increase the amount of glycogen in the liver or in the animal body, although a carbohydrate formation from glycerin, but not a glycogen formation, is probable. PFLÜGER explains this by the fact that the extent of fat metabolism is not dependent upon the quantity of fat supplied, but upon the amount of fat required by work. If more fat is supplied, then it is not destroyed, but is stored up. Even if sugar is continuously formed from the fat, in metabolism this is immediately burned and does not yield any material for the formation of the reserve substance glycogen.

Opinions in regard to the influence of the proteins are somewhat contradictory. From several investigations the conclusion has been drawn that the proteins cause an increase in the glycogen of the liver. Among these investigations must be included certain feeding experiments with boiled beef (NAUNYN) or blood-fibrin (v. MERING), and especially the very careful experiments made by E. KÜLZ on hens, with pure proteins, such as casein, seralbumin, and ovalbumin. The value of these experiments is disputed by PFLÜGER, and as a direct proof against the formation of glycogen from protein he refers to SCHÖNDORFF'S investigations when feeding carbohydrate-free protein (casein) to frogs without finding the least increase in the total glycogen. Later BLUMENTHAL and WOHLGEMUTH arrived at similar results. They found no glycogen accumulation in frogs after feeding with casein or gelatin, but did find it after feeding with ovalbumin, which contains a carbohydrate group. On the contrary, BENDIX was able to show an increase in the glycogen in dogs by feeding casein and gelatin, as well as ovalbumin, and in fact a greater increase by casein than by ovalbumin. STOOKEY[1] arrived at similar results in hens, as he found a glycogen formation after feeding casein, while he obtained no positive results after feeding glucoproteids. It seems as if the conditions in cold-blooded animals were different from those in warm-blooded ones. According to PFLÜGER, the experiments of BENDIX are not conclusive, and he doubts the formation of glycogen from protein.

Many investigators are still of the opinion that an increase in the glycogen of the liver as well as of other organs can be brought about by feeding animals with carbohydrate-free proteins. The circumstance that, as shown by PFLÜGER,[2] the glycogen by long-continued starvation does not entirely disappear from the body but is being reformed, and that

[1] Schöndorff, Pflüger's Arch., 82 and 88; Blumenthal and Wohlgemuth, Berl. klin. Wochenschr., 1901; Bendix, Zeitschr. f. physiol. Chem., 32 and 34; Stookey, Amer. Journ. of Physiol., 9.

[2] Pflüger's Arch., 119.

frogs which had starved 13 months still contained remarkably large amounts of glycogen due to this reformation (PFLÜGER), makes the formation of glycogen from protein very probable.

If the question is raised as to the action of the various bodies on the accumulation of glycogen in the liver, it must be recalled that a formation of glycogen takes place in this organ, as well as a consumption of the same. An accumulation of glycogen may be caused by an increased formation of glycogen, but also by a diminished consumption, or by both.

It is not known how the various bodies above mentioned act in this regard. Certain of them probably have a retarding action on the transformation of glycogen in the liver, while others perhaps are more combustible, and in this way protect the glycogen. Some probably excite the liver-cells to a more active glycogen formation, while others yield material from which the glycogen is formed, and are *glycogen-formers* in the strictest sense of the word. The knowledge of these last-mentioned bodies is of the greatest importance in the question as to the origin of glycogen in the animal body, and the chief interest attaches to the question: To what extent are the two chief groups of food, the proteins and carbohydrates, glycogen-formers?

The great importance of the carbohydrates in the formation of glycogen has given rise to the opinion that the glycogen in the liver is produced from sugar by a synthesis in which water separates with the formation of an anhydride (LUCHSINGER and others). This theory (*anhydride theory*) has found opponents because it neither explains the formation of glycogen from such bodies as proteins, carbohydrates, gelatin, and others, nor the circumstance that the glycogen is always the same, independent of the properties of the carbohydrate introduced, whether it is dextrogyrate or levogyrate.[1] This last circumstance does not now present any special difficulty, since we know that the simple sugars can easily be transformed into each other. It was formerly the opinion of many investigators that all glycogen is formed from protein, and that this splits into two parts, one containing nitrogen and the other being free from nitrogen; the latter is the glycogen. According to these views, the carbohydrates act only in that they spare the protein and the glycogen produced therefrom (*sparing theory* of WEISS, WOLFFBERG, and others[2]).

In opposition to this theory C. and E. VOIT and their pupils have shown that the carbohydrates are " true " glycogen-formers. After partaking of large quantities of carbohydrates, the amount of glycogen stored up in the body is sometimes so great that it cannot be covered by the

[1] See Pflüger in his Arch., **121.**

[2] In regard to these two theories, see especially Wolffberg, Zeitschr. f. Biologie, **16.**

proteins decomposed during the same time, and in these cases a glycogen formation from the carbohydrates must be admitted. According to CREMER only the fermentable sugars of the six carbon series or their di- and polysaccharides are *true glycogen-formers*. For the present, only dextrose, levulose, galactose (WEINLAND [1]), and perhaps also *d*-mannose (CREMER) are designated as true glycogen-formers. Other monosaccharides may indeed, according to CREMER influence the formation of glycogen, but they are not converted into glycogen, and hence are called only *pseudoglycogen-formers*.

The poly- and disaccharides may, after a cleavage into the corresponding fermentable monosaccharides, serve as glycogen-formers. This is true for at least cane-sugar and milk-sugar, which must first be inverted in the intestine. These two varieties of sugar, therefore, cannot, like dextrose and levulose, serve as glycogen-formers after subcutaneous injection, but reappear almost entirely in the urine (DASTRE, FR. VOIT). Maltose, which is inverted by an enzyme present in the blood, passes only to a slight extent into the urine (DASTRE and BOURQUELOT and others), and it can, like the monosaccharides, even after subcutaneous injection, be used in the formation of glycogen (FR. VOIT [2]).

The ability of the liver to form glycogen from monosaccharides has also recently been shown by K. GRUBE [3] in a very interesting and direct manner, by perfusion experiments with solutions of various carbohydrates. In these perfusion experiments on tortoise livers, dextrose produced an abundant glycogen formation, while with levulose and galactose it was less abundant. Pentoses, disaccharides, casein and amino-acids (glycocoll, alanine and leucine) were inactive while on the contrary glycerin and also formaldehyde acted as glycogen-formers.

After PAVY [4] first showed the occurrence of carbohydrate groups in ovalbumin, other investigators were able to split off glucosamine from this and other protein substances (see Chapter III), and the question arose whether the amino-sugar could serve in the formation of glycogen. The investigations carried out in this direction by FABIAN, FRÄNKEL and OFFER, CATHCART and BIAL, have shown that the glucosamine introduced into the organism is in part eliminated unchanged in the urine and has no glycogen-forming action. No definite conclusions

[1] E. Voit, Zeitschr. f. Biologie, **25**, 543, and C. Voit, *ibid.*, **28**. See also Kausch and Socin, Arch. f. exp. Path. u. Pharm., **31**; Weinland, Zeitschr. f. Biologie, **40** and **38**; Cremer, *ibid.*, **42**, and Ergebnisse der Physiol., **1**.

[2] Dastre, Arch. de Physiol. (5), **3**, 1891; Dastre and Bourquelot, Compt. rend., 98; Fritz Voit, Verhandl. d. Gesellsch. f. Morph. u. Physiol. in München, 1896, and Deutsch. Arch. f. klin. Med., **58**.

[3] Pflüger's Arch., **118**, **121**, **122** and **126**.

[4] The Physiology of the Carbohydrates, London, 1894.

can be drawn from this on the behavior of the carbohydrate groups, which exist not as free groups but combined with the protein molecules. The investigations of FORSCHBACH on the behavior of glucosamine chained to an acid-group in an amide-like combination, as well as the investigations of KURT MEYER and STOLTE,[1] have yielded no proofs for the theory that glycogen is formed from glucosamine.

Whether or not, or to what extent, the glucoproteins take part in the sugar or glycogen formation in the animal body is difficult to answer for the present, as but little is known of the quantity of these substances in the body, and our knowledge of the amount of carbohydrate which can be split off from the various protein substances is also very meagre.

If the proteins are to be counted among those bodies which can increase the glycogen of the body, then we must ask the question: Do the proteins act only indirectly as pseudoglycogen-formers, or are they direct glycogen-formers which can serve as material for the formation of glycogen or sugar? This question stands in close relation to the sugar formation and sugar elimination in the various forms of glycosuria, and will be best discussed below in connection with the question of diabetes.

Glycogen is a reserve-food deposited in the liver, and which, like other carbohydrates can be transformed into fat, and it is generally admitted that such a fat-formation from glycogen also takes place in the liver. There is no doubt that the glycogen deposited in the liver is formed in the liver-cells from the sugar; but where does the glycogen existing in the other organs, such as the muscles, originate? Is the glycogen of the muscles formed on the spot or is it transmitted to the muscles by the blood? These questions cannot at present be answered with positiveness, and the investigations on this subject by different experimenters have given varying results. The experiments of KÜLZ,[2] in which he studied the glycogen formation by passing blood containing cane-sugar through the muscle, have led to no conclusive results, while the perfusion experiments of HATCHER and WOLFF with dextrose seem to indicate a glycogen formation from sugar in the muscles. The investigations of DE FILIPPI[3] on dogs with so-called Eck's fistula also show a glycogen formation from sugar in the muscles. In the Eck fistula operation the portal vein is ligated near the liver hilus and sewed to the inferior vena cava and an opening established between the two veins so that the portal blood flows

[1] Fabian, Zeitschr. f. physiol. Chem., **27**; Fränkel and Offer, Centralbl. f. Physiol., **13**; Cathcart, Zeitschr. f. physiol. Chem., **39**; Bial, Berl. klin. Wochenschr., 1905; Forschbach, Hofmeister's Beiträge, **8**; Meyer, *ibid.*, **9**; Stolte, *ibid.*, **11**.

[2] See Minkowski and Laves, Arch. f. exp. Path. u. Pharm., **23**; Külz, Zeitschr. f. Biologie, **27**; Hatcher and Wolff, Journ. of Biol. Chem., **3**.

[3] Zeitschr. f. Biol., **49** and **50**.

directly into the vena cava without passing through the liver. In well-nourished animals operated upon in this manner the livers had the same properties as those from starving animals, while on the contrary the muscles contained quantities of glycogen which corresponded to those found in a normal over-fed dog.

If it be true that the blood and lymph contain a diastatic enzyme which transforms glycogen into sugar, and also that the glycogen regularly occurs in the form-elements and is not dissolved in the fluids, it seems probable that the glycogen in solution is not transmitted by the blood to the organs, but perhaps more likely, if the leucocytes do not act as carriers, it is formed on the spot from the sugar.[1] The glycogen formation seems to be a general function of the cells. In adults, the liver, which is very rich in cells, has the property, on account of its anatomical position, of transforming large quantities of sugar into glycogen.

This glycogen, which is deposited in the liver as reserve-food, in order that it can be useful to the body, must at least in greater part be transformed into sugar and supplied to the various organs by the blood. The question now arises whether there is any foundation for the statement that the liver-glycogen is transformed into sugar.

As first shown by BERNARD and redemonstrated by many investigators, the glycogen in a dead liver is gradually changed into sugar, and this sugar formation is caused, as BERNARD supposed and ARTHUS and HUBER, PAVY, PICK and BIAL[2] proved, by a diastatic enzyme which, according to RÖHMANN and BORCHARDT,[3] is identical with a diastatic enzyme of the blood.

This post-mortem sugar formation led BERNARD to the assumption of the formation of sugar from glycogen in the liver during life. BERNARD suggested the following arguments for this theory: The liver always contains some sugar under physiological conditions, and the blood from the hepatic vein is always somewhat richer in sugar than the blood from the portal vein. BERNARD'S views found in SEEGEN an active supporter, as he tried to show by numerous experiments the physiological sugar content of the liver as well as the high sugar content of the blood of the liver veins. On the other hand the correctness of the observations of BERNARD and SEEGEN is disputed by many investigators such as PAVY, RITTER, SCHIFF, EULENBERG, LUSSANA, MOSSE, N. ZUNTZ and others,[4] and in regard to the sugar content in the two kinds of

[1] See Dastre, Compt. rend. de soc. biol., 47, 280, and Kaufmann, *ibid.*, 316.

[2] Arthus and Huber, Arch. de Physiol. (5), 4, 659; Pavy, Journal of Physiol., 22; Pick, Hofmeister's Beitr., 3; Bial. Arch. f. (Anat. u.) Physiol., 1901.

[3] Röhmann, Verh. d. Ges. deutsch. Naturf. u. Aerzte, Breslau, 1903; Borchardt, Pflüger's Arch., 100.

[4] In regard to the literature on sugar formation in the liver see Bernard, Leçons sur

blood we have come to the general conclusion that when only the stasis and other disturbing influences of the operation are prevented, the blood of the liver veins, if at all, is only slightly richer in sugar than the blood of the portal vein.[1]

The circumstance that the blood-sugar rapidly sinks to $\frac{1}{2}$–$\frac{1}{3}$ of its original quantity, or even disappears when the liver is cut out of the circulation, indicates a vital formation of sugar in the liver (Seegen, Bock and Hoffmann, Kaufmann, Tangl and Harley, Pavy). In geese whose livers were removed from the circulation, Minkowski found no sugar in the blood after a few hours. On removing the liver from the circulation by tying all the vessels to and from the organ, the quantity of sugar in the blood is not increased (Schenck[2]). An important proof of the possibility of a vital formation of sugar from the liver glycogen lies in the fact that we shall learn below of certain poisons and operative changes which may cause an abundant elimination of sugar, but only when the liver contains glycogen.

A vital formation of sugar from the liver glycogen is now generally accepted. Most investigators consider this as an enzymotic transformation of the glycogen by means of the liver diastase, while certain investigators such as Dastre, Noël-Paton, E. Cavazzani, McGuigan and Brooks[3] and others explain it by a special activity of the protoplasm.

The relation of the sugar eliminated in the urine under certain conditions, such as in diabetes mellitus, certain intoxications, lesions of the nervous system, etc., to the glycogen of the liver is also an important question.

It does not enter into the plan and scope of this book to discuss in detail the various views in regard to glycosuria and diabetes. The appearance of dextrose in the urine is a symptom which may have essentially different causes, depending upon different circumstances. Only a few of the most important points will be mentioned.

The blood always contains about the average of 1 p. m., while the urine has in it at most only traces of dextrose. When the quantity of sugar in the blood rises to 3 p. m. or above, then sugar passes into the urine, but not always.[4] The kidneys have the property to a certain

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le diabète, Paris, 1877; Seegen, Die Zuckerbildung im Tierkörper, 2. Aufl., Berlin, 1900; M. Bial, Pflüger's Arch., **55**, 434.

[1] Seegen, Die Zuckerbildung, etc., and Centralbl. f. Physiol., **10**, 497 and 822; Zuntz, *ibid.*, 561; Mosse, Pflüger's Arch., **63**; Bing, Skand. Arch. f. Physiol., **9**.

[2] Seegen, Bock, and Hoffmann, see Seegen, l. c.; Kaufmann, Arch. de Physiol. (5), **8**; Tangl and Harley, Pflüger's Arch., **61**; Pavy, Journ. of Physiol., **29**., Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Schenck, Pflüger's Arch., **57**.

[3] McGuigan and Brooks, Amer. Journ. of Physiol., **18**. In regard to the literature see Pick, Hofmeister's Beiträge, **3**, 182.

[4] See Mohr, Zeitschr. f. exp. Path. u. Therap., **4**.

extent of preventing the passage of blood-sugar into the urine; and it follows from this that an elimination of sugar in the urine may be caused partly by a reduction or suppression of this above-mentioned activity, and partly also by an abnormal increase of the quantity of sugar in the blood.

The first seems, according to v. MERING and MINKOWSKI, to be the case in phlorhizin diabetes. v. MERING found that a strong glycosuria appears in man and animals on the administration of the glucoside phlorhizin. The sugar eliminated is not derived from the glucoside alone. It is formed in the animal body, and in fact from the carbohydrates, or as generally admitted on prolonged starvation, from the protein substances of the body (LUSK). The quantity of sugar in the blood is not increased, but rather diminished, in phlorhizin diabetes (MINKOWSKI), but this is disputed by PAVY. This last investigator found, although only to a slight degree, that the sugar in the blood was increased, but he holds the same view that v. MERING does, that phlorhizin diabetes is a kidney diabetes. The fact that after extirpation of the kidney in phlorhizin diabetes no rise in the blood-sugar is observed, and that after the injection of phlorhizin in the renal artery of one side the urine secreted by this kidney contains sugar sooner and more abundantly than the urine from the other kidney (ZUNTZ), tends to favor this view. The experiments especially performed by PAVY, BRODIE, and SIAU[1] upon blood containing phlorhizin and surviving kidneys also indicate the same, namely, that the phlorhizin acts upon the kidneys. While v. MERING believes in an increased permeability of the kidneys for sugar, produced by the phlorhizin, PAVY is, on the contrary, of the opinion that the kidneys, under the influence of the phlorhizin, split off sugar from a substance circulating in the blood, perhaps from a protein with loosely combined carbohydrate groups.

Another form of glycosuria which seems to be connected with a changed permeability of the kidneys is the glycosuria first observed by BOCK and HOFFMANN after the intravascular injection of large quantities of a 1-per cent salt solution, which is also of great interest because, as shown by MARTIN FISCHER, it can be again arrested by an injection of

[1] In regard to the literature on phlorhizin diabetes see v. Mering, Zeitschr. f. klin. Med., **14** and **16**; Minkowski, Arch. f. exp. Path. u. Pharm., **31**; Moritz and Prausnitz, Zeitschr. f. Biologie, **27** and **29**; Külz and Wright, *ibid.*, **27**, 181; Cremer and Ritter, *ibid.*, **28** and **29**; Contejean, Compt. rend. de soc. biol., **48**; Lusk, Zeitschr. f. Biologie, **36** and **42**: Levene, Journal of Physiol., **17**; Pavy, *ibid.*, **20**, and with Brodie and Siau, **29**; Arteaga, Amer. Journ. of Physiol., **6**; O. Loewi, Arch. f. exp. Path. u. Pharm., **47**; N. Zuntz, Arch. f. (Anat. u.) Physiol., 1895; Stiles and Lusk, Amer. Journ. of Physiol., **10**; Lusk, *ibid.*, **22**; Cremer, Ergebnisse der Physiol., **1**, Abt. **1**, and the monographs upon diabetes.

a salt solution containing $CaCl_2$. According to the investigations of UNDERHILL and CLOSSON [1] on rabbits, the injection of salt into the carotid artery brings about a hyperglycæmia by a disturbance in respiration; the injection of the salt solution into the ear vein causes on the contrary a glycosuria with polyuria and a hypoglycæmia; and they account for the salt-glycosuria produced in this manner by pointing to an increased permeability of the kidneys.

With the exception of these two forms of glycosuria, the phlorhizin diabetes and the salt-glycosuria, and also the glycosuria produced by uranium salts, all other forms of glycosuria or diabetes, as far as known at present, depend on a *hyperglycæmia*.

A hyperglycæmia may be caused in various ways. It may be caused, for example, by the introduction of more sugar than the body can destroy.

The ability of the animal body to assimilate the different varieties of sugar has naturally a limit. If too much sugar is introduced into the intestinal tract at one time, so that the so-called assimilation limit (see Chapter IX, on absorption) is overreached, then the excess of absorbed sugar passes into the urine. This form of glycosuria is called *alimentary glycosuria*,[2] and it is caused by the passage of more sugar into the blood than the liver and other organs can destroy.

As the liver cannot transform into glycogen all the sugar which comes to it in these, to a certain extent physiological, alimentary glycosurias, it is possible that a glycosuria may also be produced under pathological conditions, even by a moderate amount of carbohydrate (100 grams dextrose), which a healthy person could overcome. This is true, among other cases, in various affections of the cerebral system and in certain chronic poisonings. Certain observers include the lighter forms of diabetes, where the sugar disappears from the urine when the carbohydrates are cut off as much as possible for the food, to this class of glycosuria.

A hyperglycæmia which passes into a glycosuria may also be brought about by an excessive or sudden formation of sugar from the glycogen and other substances within the animal body.

To this group of glycosurias belongs, it seems, the *adrenalin glycosuria*, in which an increased mobilization of the carbohydrate (glycogen) occurs. The so-called *piqûre* of BERNARD, and probably also those glycosurias

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[1] Bock and Hoffmann, Arch. f. (Anat. u.) Physiol., 1871; M. Fischer, University of California publications Physiol., 1903 and 1904, and Pflüger's Arch., **106** and **109**; Underhill and Closson, Amer. Journ. of Physiol., **15**, and Journ. of Biol. Chem., **4**.

[2] In regard to alimentary glycosuria see Moritz, Arch. f. klin. Med., **46**, which also contains the earlier literature; B. Rosenberg, Ueber das Vorkommen der alimentären Glykosurie, etc. (Inaug.-Dissert. Berlin, 1897); van Oondt, Münch. med. Wochenschr., 1898; v. Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

which occur after other lesions of the nervous system, belong to the above group of glycosurias. The glycosuria produced on poisoning with carbon monoxide, ether, chloroform, curare, strychnine, morphine, piperidine, etc., also belongs to this group. How these glycosurias are brought about is not known with certainty, and the conditions become complicated because in most cases the appearance of the glycosuria is connected with an insufficient supply of oxygen. UNDERHILL has shown for the piperidine-glycosuria and PENZOLDT and FLEISCHER and SAUER [1] for the curare-glycosuria that one can prevent the appearance of the glycosuria (on poisoning with piperidine) and the hyperglycæmia as well by supplying oxygen. MACLEOD [2] has also shown that the irritation of the central end of the cut vagus, or irritation of the spinal marrow, produces glycosuria and hyperglycæmia only with simultaneous dyspnœa; with sufficient oxygen supply the glycosuria remains absent. This does not conflict with the ordinary assumption that in this group of glycosurias an increased formation of sugar occurs from the glycogen. The investigations of BANG, LJUNGDAHL and BOHM,[3] in which the extent of enzymotic decomposition of glycogen in the liver was determined, also indicate positively that in the *piqûre* as well as in the asphyxia of strychnine and morphine poisoning an increased decomposition of glycogen takes place.

The material from which the sugar is formed is glycogen in most cases. That the glycosuria produced after *piqûre* is due to an increased transformation of the glycogen follows from the fact that no glycosuria appears, under the above-mentioned circumstances, when the liver has been previously made free from glycogen by starvation or other means. In other cases, as in carbon-monoxide poisoning, the origin of the sugar is less clear. In the last-mentioned case a sugar formation from proteins has indeed been accepted, as this glycosuria appears only in those cases when the poisoned animal has a sufficient quantity of protein at its disposal (STRAUB and ROSENSTEIN [4]). Protein starvation with a simultaneously abundant supply of carbohydrates causes this glycosuria to disappear. In the glycosuria produced by irritation of the vagus, which as above remarked, according to MACLEOD only appears with insufficient supply of oxygen, the hyperglycæmia (in rabbits)

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[1] Underhill, Journ. of biol. Chem., **1**; Penzoldt and Fleischer, Virchow's Arch., **87**; Sauer, Pflüger's Arch., **49**, 425, 426.

[2] Macleod, Amer. Journ. of Physiol., **19**, with Briggs, Cleveland Med. Journ., 1907.

[3] Hofmeister's Beiträge, **9** and **10**.

[4] See Dock, Pflüger's Arch., **5**; Bock and Hoffmann, Exp. Studien über Diabetes (Berlin, 1874); Cl. Bernard, Leçons sur le diabète (Paris); T. Araki, Zeitschr. f. physiol. Chem., **15**, 351; Straub, Arch. f. exp. Path. u. Pharm., **38**; Rosenstein, *ibid.*, **40**; Pflüger, Pflüger's Arch., **96**.

depends, according to BANG[1] and collaborators, upon an increased decomposition of the glycogen of the muscles and not of the liver.

A hyperglycæmia and glycosuria may also be caused by a decreased ability of the animal to consume or to utilize the sugar or to transform it into glycogen. In this case the sugar must accumulate in the blood, and the formation of severe cases of diabetes mellitus is now generally explained by this process.

The inability of diabetics to destroy or consume the sugar does not seem to be connected with any decrease in the oxidative energy of the cells. The oxidative processes are not generally diminished in diabetes (SCHULTZEN, NENCKI and SIEBER), and this has recently been substantiated by BAUMGARTEN.[2] This latter investigator made experiments with several bodies which on account of their aldehyde nature were closely related to sugar or were cleavage or oxidation products of it, namely, glucuronic acid, *d*-gluconic acid, *d*-saccharic acid, glucosamine, mucic acid, and others, and he found that diabetics destroyed or burnt these bodies to the same extent as healthy individuals. Besides this it must be remarked that the two varieties of sugar, dextrose and levulose, which are oxidized with the same readiness, act differently in diabetics. According to KÜLZ and other investigators levulose is, contrary to dextrose, utilized to a great extent in the organism, and may, according to MINKOWSKI,[3] even cause a deposit of glycogen in the liver in animals with pancreas diabetes (see below). The combustion of protein and fat takes place as in healthy subjects, and the fat is completely burned into carbon dioxide and water. In this diabetes the ability of the cells to utilize the dextrose suffers diminution, and the explanation of this has been sought in the fact that the dextrose is not previously split before combustion.

The variation in the respiratory quotient, i.e., the relation $\frac{CO_2}{O}$, seems to show an insufficiency of the dextrose combustion in the tissues in diabetes. As will be thoroughly explained in a following chapter, this quotient is greater the more carbohydrates are burnt in the body, and it is correspondingly smaller when protein and fat are chiefly burnt. The investigations of LEO, HANRIOT, WEINTRAUD and LAVES,[4] and

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[1] Bang, Ljungdahl and Bohm, Hofmeister's Beiträge, **10**.

[2] Schultzen, Berl. klin. Wochenschr., 1872; Nencki and Sieber, Journ. f. prakt. Chem. (N. F.), **26**, 35; Baumgarten, "Ein Beitrag zur Zenntniss des Diabetes mellitus," Habilitationschrift, also Zeitschr. f. exp. Path. u. Therap., **2**, 1905.

[3] Külz, Beiträge zur Path. u. Therap. des Diabetes mellitus (Marburg, 1874), **1**; Weintraud and Laves, Zeitschr. f. physiol. Chem., **19**; Haycraft, *ibid.*; Minkowski, Arch. f. exp. Path. u. Pharm., **31**.

[4] See. v Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

others have shown that in severe cases of diabetes, in the starving condition, the low quotient is not raised after partaking of dextrose, as in healthy individuals, but that it is raised after feeding levulose, which is also of value to diabetics (WEINTRAUD and LAVES). The poverty of the organs and tissues of diabetics in glycogen indicates that it is perhaps not a diminished combustion of the dextrose which is essential, but more likely an inability of the body to transform the dextrose into glycogen or to utilize it at all.

The relation of the pancreas to diabetic glycosuria is of the greatest importance for its proper understanding.

The investigations of MINKOWSKI, v. MERING, DOMINICIS, and later of many other investigators,[1] show that a true diabetes of a severe kind is caused by the total or almost total extirpation of the pancreas of many animals, especially dogs. As in man in severe forms of diabetes, so also in dogs with pancreatic diabetes, an abundant elimination of sugar takes place even on the complete exclusion of carbohydrates from the food.

Artificial *pancreas diabetes* may indeed also in other respects present the same picture as diabetes in man but there exist important differences between these two.[2] It is generally accepted that in pancreas diabetes a diminished consumption exists, i.e., diminished utilization, which does not exclude an increased sugar formation. There are also certain investigators who explain this form of diabetes as not entirely due to a diminished combustion of sugar, but to a pathological increase in the sugar formation.

Many important observations show that a close relation exists between the liver and pancreas diabetes. PFLÜGER has also especially shown that in diabetes produced by SANDMEYER'S method (partial extirpation with subsequent destruction of the remains of the gland in the abdominal cavity, when the animal remains alive for a longer time than after total extirpation) the liver does not lose weight, although the total weight of the animal diminishes greatly, while in starvation without diabetes the liver loses weight more than the other parts of the body. PFLÜGER concludes from this that the liver in diabetes works actively, and is the most important seat of production of diabetic sugar.

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[1] See Minkowski, Untersuchungen über Diabetes mellitus nach Exstirpation des Pankreas (Leipzig, 1893); v. Noorden, Die Zuckerkrankheit (Berlin, 1901), which contains a very complete index of the literature. In regard to diabetes see also Cl. Bernard, Leçons sur le diabète (Paris); Seegen, Die Zuckerbildung im Thierkörper (Berlin, 1890), and Pflüger, Des Glykogen, 2. Aufl., 1905, and especially v. Noorden's Hamb. d. Pathol. des Stoffwechsels, 2. Aufl., 1907, Bd. 2, Chapter I.

[2] See Falta "Ueber den Eiweissumsatz beim Diabetes mellitus." Berl. klin. Wochenschr., 1908, and Zeitschr. f. klin. Med., 66.

It seems as if not only does the liver stand in close relation to pancreas diabetes, but to other organs also. PFLÜGER found that in frogs, after total extirpation of the duodenum, a strong and continuous glycosuria is the result, and HERLITZKA found the same after poisoning the central nerves of the duodenum of the frog by means of nicotine. According to PFLÜGER we can explain this glycosuria by the assumption that the pancreas has an antidiabetic action which is influenced by the nerve centers of the intestine. This relation of the duodenum to pancreas diabetes has not been generally admitted, and in warm-blooded animals (at least in dogs) the occurrence of a *duodenal diabetes* has not been shown. Still REALE and DE RENZI observed a glycosuria in dogs after duodenal resection, but others have not been able to confirm this, or at least only in part. PFLÜGER, on carefully repeating REAL'S and DE RENZI'S experiment, observed only a very slight or no glycosuria at all, and previously EHRMANN, ROSENBERG and MINKOWSKI, in an especially convincing manner, after the total extirpation of the duodenum in dogs, obtained completely negative results. The positive results of REAL and DE RENZI are explained by PFLÜGER [1] by the healing up of the intestinal tube and the disturbance produced in its neighborhood. No positive conclusion can be drawn from the glycosuria observed by GAULTIER and by ZAK after corrosion of the duodenal mucous membrane, as a glycosuria can also be produced by the corrosion of other parts of the intestine (EICHLER and SILBERGLEIT [2]). The occurrence of a duodenal glycosuria in dogs has thus far not been proven.

There seems, on the contrary, to exist a relation between pancreas diabetes and the function of the adrenals. As first shown by BLUM, adrenalin produces a strong glycosuria which apparently brings about an increase in the destruction of glycogen with hyperglycæmia by an abundant "mobilization of the carbohydrates." This glycosuric action of adrenalin could be prevented by ZUELZER by the injection of pancreas extracts, and this statement is confirmed by FRUGONI by experiments with pancreatic juice. Further proof of the relation of the adrenals to the pancreas has been given by EPPINGER, FALTA and RUDINGER.[3] According to the last-mentioned investigators there is evidence of a certain relation existing in pancreas diabetes between the pancreas, adrenals and thyroids. According to them a mutual retarding action exists between the pancreas and the thyroids as well as between the

[1] Pflüger in his Archives, **118**, **119**, **122**, **124**; Minkowski, Arch. f. exp. Path. u. Pharm., 58. The other works cited are found in the above literature.

[2] Gaultier, Compt. rend. soc. biol., **64**; Zak, Wien. klin. Wochenschr., **21**; Eichler and Silbergleit, Berlin. klin. Wochenschr., 1908.

[3] Frugoni, Berl. klin. Wochenschr., 1908; Eppinger, Falta and Rudinger, Zeitschr. f. klin. Med., **66**, which also contains the literature on adrenalin diabetes.

pancreas and the adrenals, while between the thyroids and the adrenals a mutual accelerating action exists. In depancreatized dogs the retarding action of the pancreas upon the thyroids is removed, and in this way we explain the strong increase in the protein, fat (MOHR) and salt-metabolism (FALTA and WHITNEY [1]) observed in pancreas diabetes. By the removal of the retarding action of the pancreas upon the adrenals, the mobilization of the carbohydrates by means of the adrenalin is increased, and herein, as well as the diminished sugar utilization, lies the reason for the strong elimination of sugar. The relations between the above three glands is still further described by the above-mentioned authors, but we cannot enter more into detail in regard to the interesting question, which requires further study. Nevertheless we must mention that according to PICK and PINELES [2] the extirpation of the thyroid glands in young goats, but not in rabbits, prevents the appearance of adrenalin-glycosuria. The negative results with rabbits probably depend upon the fact that in rabbits the parathyroids remain completely intact. R. HIRSCH has observed in dogs that complete thyroidectomy, but not the removal of the chief thyroid glands alone, itself brings about an alimentary glycosuria.

The conditions in pancreas diabetes are certainly very complicated, and the reasons for this are still very dark. Most investigators are of the view that we are here dealing with the abolition of one or more bodies which are considered as products of the internal secretion of the glands (*hormones* according to STARLING) and which in an unknown manner regulate the sugar destruction or carbohydrate metabolism.

The assumption of an internal secretion is based on the investigations of MINKOWSKI, HÉDON, LANCERAUX, THIROLOIX, and others [3] upon the action of the subcutaneous transplantation of the gland. According to these investigations a subcutaneously transplanted piece of the gland can completely perform the functions of the pancreas as to the sugar exchange and the sugar elimination, because on the removal of the intra-abdominal piece of gland the animal in this case does not become diabetic, but if the subcutaneously embedded piece of pancreas is subsequently removed, an active elimination of sugar appears immediately. PFLÜGER has made important objections to the value of the results of these experiments, and on the other hand ZUELZER, DOHRN and

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[1] Mohr, Zeitschr. f. exp. Path. u. Therap., **4**; Falta and Whitney, Hofmeister's Beiträge, **11**.

[2] Pick and Pineles, Bioch. Zeitschr., **12**; R. Hirsch, Zeitschr. f. exp. Path. u. Therap., **5**.

[3] See Minkowski, Arch. f. exp. Path. u. Pharm., **31**; Hédon, Diabète pancréatique, Travaux de Physiologie (Laboratoire de Montpellier, 1898), and the works on diabetes.

MARXER [1] have recently in an important work given the theory of internal secretion a strong support, if their work is substantiated. These investigators have not only given further proof of the antagonism between the adrenals and the pancreas, but they have obtained a preparation from the pancreas, in a manner not described in detail, which causes in dogs as well as in man a diminution in the elimination of sugar (and acetone bodies) in diabetes, and an improvement in the general condition.

This internal secretion of the pancreas has in recent times been supposed to be connected with the so-called islands of LANGERHANS; but no positive results have been obtained in this connection. Nor are we acquainted with the kind of active substance here formed.

The glycolytic property of the blood as shown by LÉPINE was considered for a time to be due to a glycolytic enzyme formed in the pancreas, and pancreas diabetes used to be explained by the fact that the action of this enzyme was removed when the gland was extirpated. This glycolysis is not sufficient, even if it is derived from the pancreas, to explain the transformation of the large quantity of sugar in the body, and for the destruction of sugar we are also obliged to accept a glycolysis in the organs and tissues. Opinions in regard to this glycolysis differ in certain points. According to one view (SPITZER and others) special oxidases are active in the glycolysis, while another (STOKLASA) considers the glycolysis as analogous to alcoholic fermentation, where we have processes brought on by special tissue zymases.

The prevailing opinions hold that (Chapter IV) alcoholic fermentation takes place in two steps. In the first step lactic acid is produced from the sugar and in the second the lactic acid splits into carbon dioxide and alcohol. According to STOKLASA [2] and his collaborators a decomposition of sugar occurs in the animal tissues in a similar manner by the analogous action of enzymes. Many objections have been advanced from various quarters against these investigations, which seem to indicate that in these cases the experimenters were dealing with the action of micro-organisms.[3] According to HAMMARSTEN the claims of STOKLASA and his collaborators are not disproved, and we cannot dispute the possibility that in the animal tissues as well as in the plant [4] tissues in anaerobic respiration, an alcoholic fermentation may

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[1] Deutsch. med. Wochenschr., 1908.

[2] Hofmeister's Beiträge, **3**, Centralbl. f. Physiol., **16**, **17**, **18**; Ber. d. d. chem. Gesellsch., **38**; also with Czerny, *ibid.*, **36**; with Jelinek, Simaček and Vitek, Pflüger's Arch., 101.

[3] See the works of O. Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**; Batelli, Compt. rend., **137**; Portier, Compt. rend. soc. biol., **57**.

[4] See Palladin, Zeitschr. f. physiol. Chem., **55** and **56**.

occur. Recently VAHLEN[1] found a substance in the pancreas which accelerates catalytically the alcoholic fermentation of sugar by yeast.

That lactic acid can be an intermediary step in the destruction of sugar in the animal body follows from the several circumstances as to the origin of lactic acid, which will be mentioned in a subsequent chapter (XI, Muscle,) and also the observation of A. R. MANDEL and LUSK[2] on the relation of lactic acid to diabetes. These experimenters showed after phosphorus poisoning in dogs, that the blood and urine contained abundance of lactic acid, and on producing phlorhizin-diabetes it disappeared from these fluids, and also that phosphorus poisoning does not cause a lactic acid formation in dogs with phlorhizin-diabetes. Although it is difficult to give a satisfactory interpretation of these observations, it is still very probable that in the elimination of the sugar in phlorhizin-diabetes a mother substance of the lactic acid is lost.

We are not agreed as to the ways and means which bring about the so-called glycolysis, and another disputed question is whether the glycolysis can be produced by one organ or only by the combined action of several organs. COHNHEIM found that a cell-free fluid can be obtained from a mixture of pancreas and muscle, which destroys dextrose, while the pancreas alone does not have this action, and the muscle only to a slight extent. The pancreas does not contain, according to COHNHEIM, a glycolytic enzyme, but a substance resistant to boiling temperatures, which is soluble in water and alcohol, and which, like an amboceptor, activates a glycolytic proenzyme which exists in the muscle fluid, but which is inactive alone and which retards glycolysis when it exists in excess. DE MEYER[3] holds an almost similar view, but with this exception, that he does not consider the activating substance coming from the muscles, but from the leucocytes. This proenzyme is activated by the internal secretion of the pancreas.

The findings of COHNHEIM have not been fully confirmed by other investigators.[4] Certain of these investigators come to more or less similar conclusions while, on the contrary, others cannot substantiate his deductions at all, and for the present our knowledge of the mode of action of the pancreas in the sugar metabolism in the animal body is very meager and incomplete.

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[1] Zeitschr. f. physiol. Chem., **59**.

[2] Amer. Journ. of Physiol., **16**.

[3] Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**, and **47**; De Meyer, Arch. intern. de Physiol., **2**, cited from Biochem. Centralbl., **3**, and Centralbl. f. Physiol., **20**.

[4] Stocklasa and collaborators, Centralbl. f. Physiol., **17**, and Ber. d. d. chem. Gesellsch., **36** and **38**; Feinschmidt, Hofmeister's Beiträge, **4**; Hirsch, *ibid.*; Claus and Embden, *ibid.*, **6**; Arnheim and Rosenbaum, Zeitschr. f. physiol. Chem., **40**; Braunstein, Zeitschr. f. klin. Med., **51**.

Where does the sugar eliminated in diabetes originate? Does it depend entirely upon the carbohydrates of the food or the store of carbohydrate in the body, or has the body the power of producing sugar from other material? To LÜTHJE belongs the credit for positively deciding this question. He has made experiments on dogs with pancreas diabetes, in which on a protein diet free from carbohydrates so much sugar was eliminated that it could not possibly be accounted for by the store of glycogen or other carbohydrate-containing substances in the body. Similar experiments were also performed later by PFLÜGER,[1] with the results that the power of the animal body to produce sugar from non-carbohydrate material is now definitely proven.

Is this sugar produced from protein or fat, or from both? This question so far has not been answered, and it is the subject of continuous dispute. It is not possible to enter into an exhaustive and detailed discussion of the question in a text-book, and we will only mention, briefly, certain of the most important observations and historical points.

The largest amount of sugar which we can obtain theoretically from protein is 8 grams of sugar from 1 gram of protein nitrogen if we admit that all the carbon of the protein, with the exception of that necessary to form ammonium carbonate, is used for the formation of sugar. These results are still somewhat too high for the average carbon and nitrogen content of the proteins and the values $D:N = 6.6$ is probably more correct.[2] The actual relation between dextrose and nitrogen in the urine, i.e., the quotient D:N, has been repeatedly determined in various forms of diabetes, and in depancreatized dogs it is generally 2.8 and in starving dogs or dogs fed with protein and poisoned with phlorhizin it is equal to 3.65 (LUSK). It may undergo considerable variation, and in certain cases it may indeed be lower than 1 as well as higher than 8, and high results have been repeatedly obtained in cases of human diabetes. From these quotients conclusions have been drawn as to the amount of sugar formed, as well as the origin of the sugar, but according to the views of HAMMARSTEN such conclusions are mostly very uncertain. The sugar eliminated by the urine represents the difference between the total sugar production of the body and the quantity of sugar burned or utilized. Only under the supposition that the body cannot burn or utilize any sugar is the sugar of the urine a measure of the quantity of sugar produced: it is not known how far this supposition can be applied in the various forms of diabetes. Still several observations seem to show that in the different forms of diabetes variable amounts of the sugar are burned,

[1] Lüthje, Deutsch. Arch. f. klin. Med., 79, and Pflüger's Arch., 106; Pflüger, Pflüger's Arch., 108.

[2] See Falta, Zeitschr. f. klin. Med., 65.

and only in special cases can we draw approximately accurate conclusions. When, for example, the quotient in a case was especially high, we could conclude that sugar was formed from fat; if no nitrogen retention existed, with a carbohydrate-free diet.

The property of protein of increasing the elimination of sugar is considered as an important proof of the formation of sugar from protein. In this regard those experiments are of special interest in which the diabetic animal is allowed to starve until the urine is poor in sugar or indeed free from sugar, and then by feeding with protein an abundant elimination of sugar is produced. If we do not accept the view in this case that the protein, but rather the fat, was the material from which the sugar was produced, still we must admit either of a sugar-sparing action due to protein or of a strong sugar formation from fat, incited by the protein.

A sparing in the sense that the protein is oxidized instead of the sugar, and in this manner protects it, is naturally possible only under the supposition that the body can burn at least a part of the sugar, otherwise there would be nothing to spare and nothing to protect from burning. The assumption of such an indirect action of proteins is difficult to reconcile with the common view of the inability of the body to burn sugar in diabetes. LÜTHJE [1] has communicated one experiment among others, in which a dog with pancreas diabetes, whose weight before starvation was 18 kilos, with nineteen days' starvation eliminated an average of 10.4 grams sugar for the last six days of starvation. By exclusive protein feeding the quantity of sugar per day could be raised to a maximum of 123.6 grams, and as average it was 97.5 grams for the ten protein days. The protein, therefore, had protected daily an average of 87 grams sugar from burning, which is hardly possible; and if in the diabetic animal we admit of this considerable power of burning sugar, the quotient D:N becomes valueless as a measure of the quantity of sugar formed.

If, on the contrary, we admit of an indirect action of proteins in that they incite a sugar formation from fat, perhaps by a certain very important increase in the activity of the liver, we are opposed by the great difficulty that, according to known laws of metabolism, the proteins do not raise the fat metabolism, but rather diminish it. The protein displaces a corresponding quantity of fat from the metabolism, and if the fat were the only source of sugar then in this case we would expect a diminished elimination of sugar instead of an increased one. Nevertheless the above action of protein upon sugar elimination is much more easily explained by the assumption of a sugar formation from protein than from fat.

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[1] Deutsch. Arch. f. klin. Med., 79.

The action of monamino-acids upon the carbohydrate metabolism has also given important ground for the assumption of a sugar formation from protein. That a deamidation occurs in the animal body was shown by the earlier observations of BAUMANN and BLENDERMANN. Further proofs of this were furnished by the recent investigations of NEUBERG and LANGSTEIN, where in feeding experiments with alanine they found abundance of lactic acid in urine, and P. MAYER observed glyceric acid in the urine after the subcutaneous injection of diamino-propionic acid. Finally, LANG [1] has shown that various organs in antiseptic autolysis have the power of deamidizing amides and amino-acids. As from amino-acids by deamidation it is possible to produce oxyfatty acids according to the formula $—CH.NH_2 + H_2O = —CH(OH) + NH_3$, it was interesting to test the action of amino-acids upon carbohydrate metabolism. Several investigations have been carried on with this in view, such as those of LANGSTEIN and NEUBERG, R. COHN and F. KRAUS, which have shown a very probable formation of carbohydrate under the influence of amino-acids; but the investigations of EMBDEN and SALOMON and of EMBDEN and ALMAGIA have positively shown, in a dog without a pancreas, that the amino-acids can bring about a re-formation of carbohydrate. LUSK [2] has shown the same with glutamic acid when fed to dogs poisoned with phlorhizin. It is still an open question whether the amino-acids are only indirectly active in this, or whether they form the material from which the sugar is formed. In general we consider the formation of sugar with amino-acids as intermediary bodies as very probable.

The investigations of WEINLAND [3] tend to prove a sugar formation from protein. He studied the formation of sugar in the chrysalis pulp of the Calliphora and showed that the sugar formed thereby did not originate from the fat, but that the protein was the only material from which the sugar was formed.

If we assume a formation of sugar from fat, we must differentiate between the two components of neutral fats, that is, between the glycerin and the fatty acids. A formation of sugar from glycerin can be considered as proven by the investigations of CREMER, and especially those of LÜTHJE,[4] and in the following we will discuss only the formation of sugar from the fatty acids.

[1] Baumann, Zeitschr. f. physiol. Chem., **4**; Blendermann, *ibid.*, **6**; Neuberg and Langstein, Arch. f. (Anat. u.) Physiol., 1903, Suppl.; Mayer, Zeitschr. f. physiol. Chem., **42**; Lang, Hofmeister's Beiträge, **5**..

[2] Langstein and Neuberg, l. c.; Cohn, Zeitschr. f. physiol. Chem., **28**; F. Kraus, Berl. klin. Wochenschr., 1904; Embden and Salomon, Hofmeister's Beiträge, **5** and **6**, and with Almagia, *ibid.*, **7**; Lusk, Amer. Journ. of Physiol., **22**.

[3] Zeitschr. f. Biol., **49** (N. F., 31).

[4] Cremer, Sitzungsber. d. Ges. f. Morph. u. Physiol. München, 1902; Lüthje, Deutsch. Arch. f. klin. Med., **80**.

The formation of sugar from fat seems to occur in the plant kingdom, and as the chemical processes in the animal and plant life are in principle the same, it makes the possibility of a sugar formation from fat very probable. Such an origin of sugar in the animal body is accepted by many investigators, especially by PFLÜGER and several French observers, among whom we must specially mention CHAUVEAU and KAUFMANN.[1]

When food as free from carbohydrate as possible is taken, the quotient D:N is high, i.e., higher than 8, as well as when the quantity of sugar is so large that it cannot be accounted for by the calculated protein (and carbohydrate) metabolism, then if the observations are otherwise free from error we can admit of a formation of sugar from fat. Several such cases of diabetes in man have been published (RUMPF, ROSENQVIST, MOHR, v. NOORDEN, ALLARD, FALTA and co-workers and others), and also in animals (HARTOGH and SCHUMM[2]). Although these researches are not fully conclusive, still certain of them indicate a probable formation of sugar from fat. We also have several conditions which indicate the same, namely, that in phlorhizin diabetes after the disappearance of the liver-glycogen the fat which migrates to the liver serves as material for the formation of sugar (PFLÜGER); still this is not sufficient for a positive proof.

On the other hand there are also many observations on animals and also clinical observations which oppose the theory of the formation of sugar from fat in diabetes. LUSK found in a dog with phlorhizin diabetes that the quotient D:N=3.65:1 was not changed on feeding fat, and he has recently published results of experiments[3] which show that active muscular work, which strongly increases the fat decomposition, does not change the quotient in dogs with phlorhizin diabetes. It is difficult to draw positive conclusions from these experiments, still LUSK seems to deny the formation of sugar from fat.

Attempts have been made to solve the question as to the material from which sugar is formed by the determination of the respiratory quotient and comparing this with the quotient D:N. The calculations in this direction have not led to positive results.[4] As the quotient D:N

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[1] Kaufmann, Arch. f. Physiol. (5), 8, where Chauveau's work is cited.

[2] Rumpf, Berl. klin. Wochenschr., 1899; Rosenqvist, *ibid.*; Mohr, *ibid.*, 1901; v. Noorden, Die Zuckerkrankheit, 3. Aufl., Berlin, 1901; Allard, Arch. f. exp. Path. u. Pharm., **57**; Falta and co-workers, Zeitschr. f. klin. Med., **66**; Hartogh and Schumm, Arch. f. Path. u. Pharm., **45**. See also the works of O. Loewi, *ibid.*, **47**, and Lusk, Zeitschr. f. Biologie, **42**.

[3] Amer. Journ. of Physiol., **22**.

[4] Magnus-Levy, Zeitschr. f. klin. Med., **56**; Pflüger, Pflüger's Arch., **108**; Mohr, Zeitschr. f. exp. Path. u. Therap., **4**.

is not an accurate measure of the quantity of sugar formed, and as we, as yet, do not know the quantity of oxygen necessary to form sugar from protein, HAMMARSTEN believes that it is just as impossible to conclude from the respiratory quotient that sugar is formed from the fats as from the proteins.

We have no complete proofs of a sugar formation from fat or from protein alone, nevertheless we have proofs of the possibility of a formation from both of these. There is really no objection to the assumption that the body has the power of producing sugar from protein as well as from fat. The observations on the formation of sugar or on the carbohydrate metabolism in diabetes do not give any positive explanations as to the question whether proteins are direct glycogen-formers or not.

The Bile and its Formation.

By the establishment of a biliary fistula, an operation which was first performed by SCHWANN in 1844 and which has been improved lately by DASTRE and PAWLOW[1], it is possible to study the secretion of the bile. This secretion is continuous, but with varying intensity. It takes place under a very low pressure; therefore an apparently unimportant hindrance in the outflow of the bile, namely, a stoppage of mucus in the exit, or the secretion of large quantities of viscous bile, may cause stagnation and absorption of the bile by means of the lymphatic vessels (absorption icterus).

The quantity of bile secreted in the twenty-four hours in dogs can be exactly determined. The quantity secreted by different animals varies, and the limits are 2.9–36.4 grams of bile per kilo of weight in the twenty-four hours.[2]

The reports as to the extent of bile secretion in man are few and not to be depended on. RANKE found (using a method which is not free from criticism) a secretion of 14 grams of bile with 0.44 gram of solids per kilo in twenty-four hours. NOËL-PAYTON, MAYO-ROBSON, HAMMARSTEN, PFAFF and BALCH, and BRAND[3] found a variation between 514 and 1083 cc. per twenty-four hours. Such determinations are of doubtful value, because in most cases it follows from the composition

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[1] Schwann, Arch. f. (Anat. u.) Physiol., 1844; Dastre, Arch. de Physiol. (5), **2**; Pawlow, Ergebnisse der Physiol., **1**, Abt. 1.

[2] In regard to the quantity of bile secreted in animals see Heidenhain, Die Gallenabsonderung, in Hermann's Handbuch der Physiol., **5**, and Stadelmann, Der Icterus und seine verschiedenen Formen (Stuttgart, 1891).

[3] Ranke, Die Blutvertheilung und der Thätigkeitswechsel der Organe (Leipzig, 1871); Noël-Paton, Rep. Lab. Roy. Coll. Edinburgh, **3**; Mayo-Robson, Proc. Roy. Soc., **47**; Hammarsten, Nova Act. Reg. Soc. Scient. Upsala (3), **16**; Pfaff and Balch, Journ. of Exp. Med., 1897; Brand, Pflüger's Arch., **90**.

of the collected bile that the fluid is not the result of a secretion of normal liver bile.

The quantity of bile secreted is, however, as shown by STADELMANN,[1] subject to such great variation, even under physiological conditions, that the study of those circumstances which influence the secretion is very difficult and uncertain. The contradictory statements by different investigators may probably be explained by this fact.

In starvation the secretion diminishes. According to LUKJANOW and ALBERTONI,[2] under these conditions the absolute quantity of solids decreases, while the relative quantity increases. After partaking of food the secretion increases again. The findings are very contradictory in regard to the time necessary, after partaking of food, before the secretion reaches its maximum. After a careful examination and compilation of all the existing reports, HEIDENHAIN[3] has come to the conclusion that in dogs the curve of rapidity of secretion shows two maxima, the first at the third to fifth hour and the second at the thirteenth to fifteenth hour after partaking of food. According to BARBÉRA[4] the time when the maximum occurs is dependent upon the kind of food. With carbohydrate food it is two to three hours, after protein food three to four hours, and with fat diet it is five to seven hours, after feeding.

According to earlier observations, the proteins of all the various foods cause the greatest secretion of bile, while the carbohydrates diminish the secretion, or at least excite it much less than the proteins. This coincides with the recent observations of BARBÉRA. The authorities are by no means agreed as to the action of the fats. While many older investigators have not observed any increase, but rather the reverse in the secretion of bile after feeding with fats, the researches of BARBÉRA show an undoubted increase in the secretion of bile on fat feeding, greater even than after carbohydrate feeding. According to ROSENBERG olive-oil is a strong cholagogue, a statement which, according to other investigators—MANDELSTAMM, DOYON and DUFOURT[5]—has not been proven.

As BARBÉRA has shown, a close relation exists between the bile secretion and the quantity of urea formed, as an increase in the first

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[1] Stadelmann, Der Icterus, etc., Stuttgart, 1891.

[2] Lukjanow, Zeitschr. f. physiol. Chem., **16**; Albertoni, Recherches sur la sécrétion biliaire, Turin, 1893.

[3] Hermann's Handb., **5**, and Stadelmann, Der Icterus, etc.

[4] Centralbl. f. Physiol., **12** and **16**.

[5] Barbéra, Bull. della scienz. med. di Bologna (7), **5**, Maly's Jahresber., **24**, and Centralbl. f. Physiol., **12** and **16**; Rosenberg, Pflüger's Arch., **46**; Mandelstamm, Ueber den Einfluss einiger Arzneimittel auf Sekretion und Zusammensetzung der Galle (Dissert. Dorpat, 1890); Doyon and Dufourt, Arch. de Physiol. (5), **9**. In regard to the action of various foods on the secretion of bile see also Heidenhain, l. c.; Stadelmann, Der Icterus; and Barbéra, l. c.

goes hand in hand with an increase of the latter. The bile is, therefore, according to him, a product of disassimilation, whose quantity rises and falls with the degree of activity of the liver.

The question whether there exists special medicinal bodies, so-called *cholagogues*, which have a specific excitant action on the secretion of bile, has been answered in very different ways. Many, especially the older investigators, have observed an increase in the bile secretion after the use of certain therapeutic agents, such as calomel, rhubarb, jalap, turpentine, olive-oil, etc.; while others, especially the more recent investigators, have arrived at quite opposite results. From all appearances this contradiction is due to the great irregularity of the normal secretion, which might readily cause mistakes in tests with therapeutic agents.

SCHIFF's view, that the bile absorbed from the intestinal canal increases the secretion of bile and hence acts as a cholagogue, seems to be a positively proven fact by the investigations of several experimenters.[1] Sodium salicylate is also perhaps a cholagogue (STADELMANN, DOYON and DUFOURT, WINOGRADOW [2]).

Acids, and especially, under normal conditions, hydrochloric acid, seem to be physiological excitants for bile secretion. According to FALLOISE and FLEIG the acids act upon the duodenum and the upper part of the jejunum, and the action is brought about by a secretin formation similar to the action of acids upon the secretion of pancreatic juice (see Chapter IX). According to FALLOISE [3] chloral hydrate introduced into the duodenum causes a secretion of bile in an analogous manner, by the aid of a special *chloral secretin*.

The bile is a mixture of the secretion of the liver-cells and the so-called mucus which is secreted by the glands of the biliary passages and by the mucus membrane of the gall-bladder. The secretion of the liver, which is generally poorer in solids than the bile from the gall-bladder, is thin and clear, while the bile collected in the gall-bladder is more ropy and viscous on account of the absorption of water and the admixture of "mucus," and cloudy because of the presence of cells, pigments, and the like. The specific gravity of the bile from the gall-bladder varies considerably, being in man between 1.010 and 1.040. Its reaction is alkaline to litmus. The color changes in different animals: golden yellow, yellowish brown, olive-brown, brownish green, grass-green

[1] Schiff, Pflüger's Arch., **3**. See Stadelmann, Der Icterus, and the dissertations of his pupils, especially Winteler, "Experimentelle Beiträge zur Frage des Kreislaufes der Galle" (Inaug.-Diss. Dorpat, 1892), and Gärtner, "Experimentelle Beiträge zur Physiol. und Path. der Gallensekretion" (Inaug.- Dis. Jurjew, 1893); also Stadelmann, "Ueber den Kreislauf der Galle," Zeitschr. f. Biologie, **34**.

[2] Arch. f. (Anat. u.) Physiol., 1908. See also foot-note 5, page 393.

[3] Falloise, Bull. Acad. Roy. de Belg., 1903; Fleig, *ibid.*, 1903.

or bluish green. Bile obtained from an executed person immediately after death is golden yellow or yellow with a shade of brown. Still cases occur in which fresh human bile from the gall-bladder has a green color. The ordinary post-mortem bile has a variable color. The bile of certain animals has a peculiar odor; for example, ox-bile has an odor of musk, especially on warming. The taste of bile is also different in different animals. Human as well as ox bile has a bitter taste, with a sweetish after-taste. The bile of the pig and rabbit has an intensely persistent bitter taste. On heating bile to boiling it does not coagulate. It contains (in the ox) only traces of true mucin, and its ropy properties depend, it seems, chiefly on the presence of a nucleoalbumin similar to mucin (PAIJKULL). The bile from the animals investigated by HAMMARSTEN showed a similar behavior. HAMMARSTEN [1] has, on the contrary, found a true mucin in human bile. To all appearances this mucin originates from the biliary passages, as he found it in the bile flowing from the hepatic duct, and also because the mucous membrane of the gall-bladder, according to WAHLGREN,[2] does not in man secrete any mucin, but a mucin-like nucleoalbumin.

The specific constituents of the bile are *bile-acids* combined with alkalies, *bile-pigments*, and, besides small quantities of *lecithin* and *phosphatides*, *cholesterin*, *soaps*, *neutral fats*, *urea*, *ethereal sulphuric acid*, traces of *conjugated glucuronic acids*, *enzymes* and *mineral substances*, chiefly chlorides, besides phosphates of calcium, magnesium, and iron. Traces of copper also occur.

Bile-salts. The bile-acids which thus far have best been studied may be divided into two groups, the *glycocholic* and *taurocholic acid* groups. As found by HAMMARSTEN,[3] a third group of bile-acids occurs in the shark which are rich in sulphur, and like the ethereal sulphuric acids they split off sulphuric acid on boiling with hydrochloric acid. All glycocholic acids contain nitrogen, but are free from sulphur and can be split, with the addition of water, into glycocoll (amino-acetic acid) and a nitrogen-free acid, a cholic acid. All taurocholic acids contain nitrogen and sulphur and are split, with the addition of water, into taurine and a cholic acid. The reason for the existence of different glycocholic and taurocholic acids depends on the fact that there are several cholic acids.

The conjugated bile-acid found in the shark, and called *scymnol-sulphuric acid* by HAMMARSTEN, yields as cleavage products sulphuric acid and a non-nitrogenous

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[1] Paijkull, Zeitschr. f. physiol. Chem., **12**; Hammarsten, l. c., Nova Act. (3), **16**, and Ergebnisse der Physiol., Bd. **4**.

[2] Maly's Jahserber., **32**.

[3] Hammarsten, Zeitschr. f. physiol. Chem., **24**.

substance, *scymnol* ($C_{27}H_{46}O_5$), which gives the characteristic color reactions of cholic acid.

The different bile-acids occur in the bile as alkali salts, generally the sodium compounds, even in sea-fishes, although this is contrary to the earlier observations (ZANETTI [1]). In the bile of certain animals we find almost solely glycocholic acid, in others only taurocholic acid, and in other animals a mixture of both (see below).

All alkali salts of the biliary acids are soluble in water and alcohol, but insoluble in ether. Their solution in alcohol is therefore precipitated by ether, and this precipitate, with proper care in manipulation, gives, for nearly all kinds of bile thus far investigated, rosettes or balls of fine needles or four- to six-sided prisms (PLATTNER'S crystallized bile). Fresh human bile also crystallizes readily. The bile-acids and their salts are optically active and dextrorotatory. The salts of the different bile-acids act somewhat differently toward neutral salts. The alkali salts of the ordinary and best-studied bile-acids from man, ox, and dog are, according to TENGSTRÖM,[2] precipitated by ammonium and magnesium sulphates, and also, in pure form, by sodium nitrate and sodium chloride (added to saturation). Potassium and sodium sulphates do not precipitate them. The alkali salts cannot be directly precipitated from the bile by NaCl, on account of the presence of bodies retarding precipitation, among which we find oil-soaps.

The bile-acids are dissolved by concentrated sulphuric acid at the ordinary temperature, forming a reddish-yellow liquid which has a beautiful green fluorescence. According to PREGL an oxidation with a reduction of the sulphuric acid into sulphur dioxide takes place. The fluorescent substance has been called dehydrocholan (see below) by PREGL.[3] On carefully warming with concentrated sulphuric acid and a little cane-sugar, the bile-acids give a beautiful cherry-red or reddish-violet liquid. PETTENKOFER'S reaction for bile-acids is based on this behavior.

PETTENKOFER'S *test for bile-acids* is performed as follows: A small quantity of bile in substance is dissolved in a small porcelain dish in concentrated sulphuric acid and warmed, or some of the liquid containing the bile-acids is mixed with concentrated sulphuric acid, taking special care in both cases that the temperature does not rise higher than 60–70° C. Then a 10 per cent solution of cane-sugar is added, drop by drop, continually stirring with a glass rod. The presence of bile is indicated by the production of a beautiful red liquid, whose color does not disappear at the ordinary temperature, but becomes more bluish violet in

[1] See Chem. Centralbl., 1903, **1**, 180.
[2] Zeitschr. f. physiol. Chem., **41**.
[3] Zeitschr. f. physiol. Chem., **45**.

the course of a day. This red liquid shows a spectrum with two absorption-bands, the one at *F* and the other between *D* and *E*, near *E*.

This extremely delicate test fails, however, when the solution is heated too high or if an improper quantity—generally too much—of the sugar is added. In the last-mentioned case the sugar easily carbonizes and the test becomes brown or dark brown. The reaction fails if the sulphuric acid contains sulphurous acid or the lower oxides of nitrogen. Many other substances, such as proteins, oleic acid, amyl alcohol, and morphine, give a similar reaction, and therefore in doubtful cases the spectroscopic examination of the red solution must not be forgotten.

PETTENKOFER'S test for the bile-acids depends essentially on the fact that furfurol is formed from the sugar by the sulphuric acid, and this body can therefore be substituted for the sugar in this test (MYLIUS). According to MYLIUS and v. UDRANSZKY[1] a 1 p. m. solution of furfurol should be used. Dissolve the bile, which must first be decolorized by animal charcoal, in alcohol. To each cubic centimeter of alcoholic solution of bile in a test-tube add 1 drop of the furfurol solution and 1 cc. concentrated sulphuric acid, and cool when necessary, so that the test does not become too warm. This reaction, when performed as described, will detect $\frac{1}{20}$ to $\frac{1}{80}$ milligram cholic acid (v. UDRANSZKY). Other modifications of PETTENKOFER'S test have been proposed.

The assumption that PETTENKOFER'S reaction is due to the production of furfurol from the sugar is not sufficiently proven, and according to certain investigators, such as BARDACHZI and VILLE[2] the spectrum is somewhat different when using furfurol.

Glycocholic Acid. The constitution of the glycocholic acid occurring in human and ox bile, and which has been most studied, is represented by the formula $C_{26}H_{43}NO_6$. Glycocholic acid is absent, or nearly so, in the bile of carnivora. On boiling with acids or alkalies this acid, which is analogous to hippuric acid, is converted into cholic acid and glycocoll.

By the action of hydrazine hydrate upon the ethyl ester of cholic acid BONDI and MÜLLER[3] prepared first cholic-acid hydrazide, and then, by the action of nitrous acid upon this, they obtained the cholic-acid azide, $C_{23}H_{39}O_3CO.N_3$, and finally from this last in alkaline solution with glycocoll they synthetically prepared the alkali salt of glycocholic acid, at the same time splitting off nitrogen.

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[1] Mylius, Zeitschr. f. physiol. Chem., **11**; v. Udranszky, *ibid.*, **12**.

Bardachzi, Zeitschr, f. physiol. Chem., **48**; Ville, cited from Chem. Centralbl., 1907, **2**, p. 1712

Zeitschr f. physiol. Chem., **47**.

Glycocholic acid crystallizes in fine, colorless needles or prisms. It is soluble with difficulty in water (in about 300 parts cold and 120 parts boiling water), and is easily precipitated from its alkali-salt solution by the addition of dilute mineral acids. According to BONDI[1] glycocholic acid is a rather strong acid, about as acid as lactic but much stronger than acetic acid. This last-mentioned acid precipitates glycocholic acid from the solution of its alkali salts in water. It is readily soluble in strong alcohol, but with great difficulty in ether. The solutions have a bitter but at the same time sweetish taste. The acid melts between 132–152°, depending upon the method of preparation. EMICH[2] found the melting-point 132–134° for the acid crystallized out of water. The salts of the alkalies and alkaline earths are soluble in alcohol and water.

The solution of the alkali salt in water can be salted out by NaCl, but not by KCl. The salts of the heavy metals are mostly insoluble or soluble with difficulty in water. The solution of the alkali salts in water is precipitated by sugar of lead, cupric and ferric salts, and silver nitrate.

Glycocholeic Acid is a second glycocholic acid, first isolated by WAHLGREN[3] from ox-bile, and has the formula $C_{26}H_{43}NO_5$ or $C_{27}H_{45}NO_5$. This acid, which on hydrolytic cleavage yields glycocoll and choleic acid, has also been detected in human bile and the bile of the musk-ox (HAMMARSTEN[4]).

Glycocholeic acid may, like glycocholic acid, crystallize in tufts of fine needles, but is often obtained as short thick prisms. It is much more insoluble in water, even on boiling, than glycocholic acid, and it melts at 175–176° C. The alkali salts are soluble in water, have a pure bitter taste, and are more readily precipitated by neutral salts (NaCl) than the glycocholates. The solution of the alkali salts is not only precipitated by the salts of the heavy metals, but also by the salts of barium, calcium and magnesium.

The principle of the preparation of the pure glycocholic acids consists in treating a 2–3 per-cent solution of bile free from mucus, when rich in glycocholic acid (so-called HÜFNER's bile[5]), with ether, and then with 2-per cent hydrochloric acid. If the bile is not directly precipitable with hydrochloric acid (bile relatively poor in glycocholic acid) then precipitate the chief mass of the glycocholic acid with ferric chloride, or better with lead acetate, decompose the precipitate with soda and treat the 2-per cent solution as above stated with ether and hydrochloric acid.

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[1] Zeitschr. f. physiol. Chem., **53**.
[2] Monatsh. f. Chem., **3**.
[3] Zeitschr. f. physiol. Chem., **36**.
[4] *Ibid.*, **43**.
[5] Hüfner, Journ. f. prakt. Chem. (N. F.), **10**, **19**, and **25**.

The crystalline and washed mass is boiled with water, and on cooling glycocholic acid crystallizes out, and then this is recrystallized from water or from alcohol by the addition of water. The residue that remains after boiling in water (paraglycocholic acid and glycocholeic acid) is converted into their barium salts, and after a complicated method (see WAHLGREN) the glycocholeic acid is obtained. The reader is referred to more exhaustive works for other methods of preparation.

Hyoglycocholic Acid, $C_{27}H_{43}NO_5$, is the crystalline glycocholic acid obtained from the bile of the pig. It is very insoluble in water. The alkali salts, whose solutions have an intensely bitter taste, without any sweetish after-taste, are precipitated by $CaCl_2$, $BaCl_2$, and $MgCl_2$, and may be salted out like a soap by Na_2SO_4 when added in sufficient quantity. By precipitation with NaCl in such quantity that the precipitate redissolves on warming, HAMMARSTEN [1] obtained the alkali salt as macroscopic crystals on cooling. Besides this acid there occurs in the bile of the pig still another glycocholic acid (JOLIN [2]).

The glycocholate in the bile of rodents is also precipitated by the above-mentioned earthy salts, but cannot, like the corresponding salt in human or ox bile, be directly precipitated on saturating with a neutral salt (Na_2SO_4). **Guano bile-acid** possibly belongs to the glycocholic-acid group, and is found in Peruvian guano, but has not been thoroughly studied.

Taurocholic Acid. This acid, which is found in the bile of man, carnivora, oxen, and a few other herbivora, such as sheep and goats, has the constitution $C_{26}H_{45}NSO_7$. On boiling with acids and alkalies it splits into cholic acid and taurine. Taurocholic acid has also been prepared synthetically by BONDI and MÜLLER, using the same method as they used for glycocholic acid.

Taurocholic acid can be readily obtained, by the method suggested by HAMMARSTEN,[3] as groups of fine needles or as beautiful prisms on slow crystallization. The crystals do not change in the air, but they decompose above 100°. They are soluble in alcohol but insoluble in ether, benzene, and acetone. Taurocholic acid is very soluble in water, and the solution has a very sweet taste, with only a slight bitter taste. It can hold the difficultly soluble glycocholic acid in solution. This is the reason why a mixture of glycocholate with a sufficient quantity of taurocholate, which often occurs in ox-bile, is not precipitated by a dilute acid. Its salts are, as a rule, readily soluble in water, and the solutions of the alkali salts are not precipitated by copper sulphate, silver nitrate or lead acetate. Basic lead acetate gives, on the contrary, a precipitate which is soluble in boiling alcohol. The alkali salts are not only precipitated from their solution by the same neutral salts that precipitate glycocholic acid, but also by potassium chloride, and by sodium and potassium acetates.

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[1] Not published.

[2] Zeitschr. f physiol. Chem., **12** and **13**.

[3] Zeitschr. f. physiol. Chem., **43**.

Taurocholeic Acid is a second taurocholic acid, detected by HAMMARSTEN in dog-bile and isolated by GULLBRING [1] from ox-bile, and has the formula $C_{26}H_{45}NSO_6$ or $C_{27}H_{47}NSO_6$. Thus far it has been obtained only in the amorphous form. It is readily soluble in water, and has a disagreeably bitter taste. It is also readily soluble in alcohol, but insoluble in ether, acetone, chloroform, and benzene. The alkali salt, soluble in water, can be salted out by NaCl as a pasty mass. The solutions of the salts can be precipitated by ferric chloride. The cleavage products are taurine and choleic acid.

The taurocholic acids are most simply prepared from bile, free from glycocholic acid or poor therein, such as fish- or dog-bile, easiest from the latter. The aqueous solution of the mucus-free bile is almost completely precipitated by ferric chloride. The precipitate is worked for taurocholeic acid and the filtrate for taurocholic acid. The iron is first removed from the filtrate by Na_2CO_3, and then the faintly alkaline filtrate saturated with NaCl. The taurocholate separates out and after further purification is decomposed by alcohol containing hydrochloric acid. The taurocholic acid is precipitated from the alcoholic filtrate by ether and recrystallized from alcohol containing water by the addition of ether. The taurocholeic acid is obtained from the above iron precipitate by treating it with soda, and decomposing the alkali salt of the taurocholeic acid with alcohol, containing HCl, and precipitating the acid from the alcoholic solution with ether and repeating this precipitation from alcohol by ether.

Cheno-taurocholic Acid. This is the most essential acid of goose-bile and has the formula $C_{29}H_{49}NSO_6$. This acid, but little studied, is amorphous and soluble in water and alcohol.

The taurocholic acids differ from the glycocholic acids in being readily soluble in water. In the bile of the walrus, on the contrary, a relatively insoluble, readily crystallizable taurocholic acid occurs which can be precipitated from the solution of the alkali salts by the addition of mineral acids, like glycocholic acid (HAMMARSTEN [2]).

As repeatedly mentioned above, the two bile-acids split on boiling with acids or alkalies into non-nitrogenous cholic acids and glycocoll or taurine. Of the various cholic acids the following have been best studied:

Cholic Acid or Cholalic Acid. The ordinary cholic acid obtained as a decomposition product of human and ox bile, which occurs regularly in the contents of the intestine, and also in the urine in icterus, has, according to STRECKER and nearly all recent investigators, the constitution

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[1] Hammarsten, Zeitschr. f. physiol. Chem., 43; Gullbring, *ibid.*, 45.

[2] Not published.

$C_{24}H_{40}O_5$, $= C_{20}H_{31}\begin{cases}CHOH\\(CH_2OH)_2\\COOH\end{cases}$. According to MYLIUS,[1] cholic acid is a monobasic alcohol-acid with one secondary and two primary alcohol groups. CURTIUS[2] has shown by preparing the cholamine, $C_{23}H_{39}O_3.NH_2$, from the above-mentioned (p. 397) cholic-acid azide, with cholic-acid urethane as an intermediary step, that the carboxyl group is not immediately connected with the CHOH group, but is combined with the chief nucleus without the neighboring secondary alcohol group. On oxidation it first yields *dehydrocholic acid*, $C_{24}H_{34}O_5$ (HAMMARSTEN). On further oxidation *bilianic acid*, $C_{24}H_{34}O_8$ (CLEVE), is obtained, or, more correctly, according to LATSCHINOFF, LASSAR-COHN and PREGL, a mixture of bilianic and *isobilianic* acids discovered by LATSCHINOFF. On oxidation, bilianic acid yields *cilianic acid* (LASSAR-COHN), whose formula, according to PREGL,[3] is $C_{20}H_{28}O_8$. On reduction (by putrefaction) MYLIUS obtained desoxycholic acid, $C_{24}H_{40}O_4$ from cholic acid.

On stronger oxidation it yields *cholesterinic acid*, which has not been carefully studied, and finally phthalic acid, as maintained by SÉNKOWSKI, but not substantiated by BULNHEIM or PREGL.[4] On reduction with hydriodic acid and red phosphorus, PREGL obtained a product which he considers as a mono-carboxylic acid, with the formula $C_{20}H_{31}\begin{cases}CH_2\\(CH_3)_2\\COOH\end{cases}$. SÉNKOWSKI[5] obtained an acid with the formula $C_{24}H_{40}O_2$, *cholylic acid*, on the reduction of the anhydride.

As above-mentioned, PREGL[6] obtained, by the action of concentrated sulphuric acid upon cholic acid, a fluorescent substance which he calls *dehydrocholon*. This is produced by oxidation, and at the same time, water is eliminated. It has probably the formula $C_{24}H_{38}O$. Dehydrocholon is nitrated by nitric acid, while the cholic acid is not. From this behavior, as well as from the determination of the molecular refraction and dispersion of both bodies, PREGL finds it probable that cholic acid belongs to the hydrated carbocyclic compounds. This view has received further support by PANZER,[7] who obtained a homologue of benzene having the formula $C_{11}H_{16}$ from a cholic acid derivate, the chole-

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[1] The important researches of Strecker on the bile-acids may be found in Annal. d. Chem. u. Pharm., **65**, **67**, and **70**; Mylius, Ber. d. deutsch. chem. Gesellsch., **19**.

[2] *Ibid.*, 39.

[3] Hammarsten, Ber. d. deutsch. chem. Gesellsch., **14**; Cleve, Bull. Soc. chim., **35**; Latschinoff, Ber. d. d. chem. Gesellsch., **15**; Lassar-Cohn, Ber. d. d. chem. Gesellsch., **32**; Pregl, Wien. Sitzungsber., **111**, 1902.

[4] Sénkowski, Monatsh. f. Chem., **17**; Bulnheim, Zeitschr. f. physiol. Chem., **25** which also contains the literature on cholesterinic acid.

[5] Mylius, l. c.; Pregl, Pflüger's Arch., **71**; Sénkowski, Monatshefte f. Chem., 19.

[6] Zeitschr. f. physiol. Chem., **45**.

[7] Zeitschr. f. physiol. Chem., **48**; Latschinoff, Ber. d. d. Chem. Gesellsch., **12** and **13**

campheric acid of LATSCHINOFF, by distillation with soda-lime and which can be considered as formed from a hydro-aromatic ring by splitting off of water and formation of double bonds.

Cholic acid crystallizes partly in rhombic plates or prisms with one molecule of water and partly in larger rhombic tetrahedra or octahedra with one molecule of alcohol of crystallization (MYLIUS). These crystals quickly become opaque and porcelain-white in the air. They are quite insoluble in water (in 4000 parts cold and 750 parts boiling), rather soluble in alcohol, but soluble with difficulty in ether. The amorphous cholic acid is less insoluble. The solutions have a bitter-sweetish taste. The crystals lose their alcohol of crystallization only after a lengthy heating to 100–120° C. The acid free from water and alcohol melts at 195° C. According to BONDI and MÜLLER the melting-point of the perfectly pure acid is 198°. It forms a characteristic blue compound with iodine (MYLIUS). If finely powdered cholic acid is added to 25-per cent hydrochloric acid at the ordinary temperature, a beautiful violet-blue coloration gradually appears, and this color is permanent for some time and then becomes gradually green and yellow. The blue solution shows an absorption band in the neighborhood of the D line (HAMMARSTEN).

The alkali salts are readily soluble in water, but when treated with a concentrated caustic or carbonated alkali solution they may then be separated as an oily mass which becomes crystalline on cooling. The alkali salts are not readily soluble in alcohol, and on the evaporation of the alcohol they may crystallize. The specific rotatory power of the sodium salt [1] is $(\alpha)_D = +30.61°$ (2.29-per cent concentration) to $+27.46°$ (7.59-per cent concentration). The watery solution of the alkali salts, when not too dilute, is precipitated immediately or after some time by lead acetate or by barium chloride. The barium salt crystallizes in fine, silky needles, and is rather insoluble in cold, but somewhat easily soluble in warm, water. The barium salt, as well as the lead salt, which is insoluble in water, is soluble in warm alcohol.

Choleic Acid ($C_{25}H_{42}O_4$, LATSCHINOFF) is another cholic acid which, according to LASSAR-COHN,[2] has the formula $C_{24}H_{40}O_4$. This acid, which occurs in varying but always small quantities in ox-bile, yields *dehydrocholeic acid*, $C_{24}H_{34}O_4$, and then *cholanic acid* $C_{24}H_{34}O_7$, and *isocholanic acid* on oxidation.

Choleic acid crystallizes when free from water in hexagonal vitreous prisms with pointed ends, melting at 185–190° C. The crystalline acid containing water melts at 135–140° C. (LATSCHINOFF). The acid

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[1] See Vahlen, Zeitschr. f. physiol. Chem., **21**.

[2] Latschinoff, Ber. d. deutsch. chem. Gesellsch., **18** and **20**; Lassar-Cohn, *ibid.*, **26**, and Zeitschr. f. physiol. Chem., **17**. See also Vahlen, Zeitschr. f. physiol. Chem., **23**.

dissolves in water with difficulty and is also relatively difficultly soluble in alcohol. It has an intensely bitter taste and gives the MYLIUS iodine reaction for cholic acid, and also the color reaction of cholic acid with hydrochloric acid. The specific rotation is $(\alpha)_D = +48.87°$ (VAHLEN). The barium salt which crystallizes from the hot alcoholic solution as spherical aggregations of radial needles is more difficultly soluble in water than the corresponding cholate.

Desoxycholic Acid, $C_{24}H_{40}O_4$, is the name given by MYLIUS to a cholic acid isolated by him from putrid ox-bile, and which is formed from the cholic acid (on the putrefaction of the bile) by reduction. This last is still very improbable, and the investigations of EKBOM do not support such an assumption. On using perfectly pure cholic acid he was able to regain it almost quantitatively after the action of metallic sodium on the alcoholic solution of the acid or of zinc and alkali. By treatment with zinc and glacial acetic acid a reaction took place, but the product was a mixture of mono- and diacetyl derivatives. The observation of PREGL that desoxycholic acid, like choleic acid, yields dehydrocholeic acid and cholanic acid as oxidation products, makes the formation of desoxycholic acid from cholic acid by reduction very improbable. The conclusion of LATSCHINOFF that both choleic and desoxycholic acids are identical, is not to be accepted on account of the different properties of the two acids, and what is more probable is that we have two different acids which are probably isomeric with each other. Both acids can also be detected in perfectly fresh ox-bile as shown by LANGHELD, and also by HAMMARSTEN.[1] According to PREGL and to HAMMARSTEN, desoxycholic acid is a preformed acid of the fresh bile.

The acid crystallizes from glacial acetic acid in needles with 1 molecule acetic acid, having a melting-point of 144–145°. The melting-point of the acid crystallized from alcohol-ether is 135–155°,[2] and for the anhydrous acid or crystallized from acetone it is 172–173°. It is soluble with difficulty in water, more readily soluble in alcohol, but somewhat less soluble in glacial acetic acid than choleic acid. It has an intensely bitter taste. The acid does not give a blue iodine compound, and no color reaction with hydrochloric acid. Its barium salt is soluble with difficulty in cold water, but dissolves in boiling alcohol and crystallizes on cooling.

The cholic acids are best prepared from ox bile, which is boiled for 24 hours with 5–10-per cent caustic soda. The crude acid is precipitated

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[1] Mylius, Ber. d. d. chem. Gesellsch., **19** and **20**; Ekbom, Zeitschr. f. physiol. Chem., **50**; Pregl, Wien. Sitz.-Ber., **111** Math. Naturw. Kl., 9102; Latschinoff, Ber. d. d. chem. Gesellsch., **20**; Langheld, *ibid.*, **41**; Hammarsten, unpublished investigations.

[2] See Pregl, l. c.

by hydrochloric acid, dissolved in ammoniacal water and precipitated by $BaCl_2$. The precipitate contains essentially choleic and desoxycholic acids, while the filtrate contains a part of these and the chief part of the cholic acid. In regard to the further rather complicated method of separating the various acids, as also in regard to the many methods suggested for the preparation of the pure cholic acids, we must refer to completer hand-books.

Fellic Acid, $C_{23}H_{40}O_4$ is a cholic acid, so called by SCHOTTEN, which he obtained from human bile, along with the ordinary acid. This acid is crystalline, is insoluble in water, and yields barium and magnesium salts, which are very insoluble. It does not respond to PETTENKOFER'S reaction easily and gives a more reddish-blue color. The existence of this acid is still doubtful.

The conjugate acids of human bile have not been sufficiently investigated. To all appearances human bile contains under different circumstances various conjugate bile-acids. In some cases the bile-salts of human bile are precipitated by $BaCl_2$ and in others not. According to the statements of LASSAR-COHN[1] three cholic acids may be prepared from human bile, namely, ordinary CHOLIC ACID, CHOLEIC ACID, and FELLIC ACID.

Lithofellic Acid, $C_{20}H_{36}O_4$, is the acid related to cholic acid which occurs in the oriental bezoar stones, which is insoluble in water, comparatively easily soluble in alcohol, but only slightly soluble in ether.[2]

The hyo-glycocholic and cheno-taurocholic acids, as well as the glycocholic acid of the bile of rodents, yield corresponding cholic acids. This also seems to be the case with the glycocholic acid of the hippotamus-bile, which stands very close to the pig-bile (HAMMARSTEN[3]). In the polar bear a third cholic acid exists besides cholic and choleic acids. It is called *ursocholeic acid*, $C_{19}H_{30}O_4$ or $C_{18}H_{82}O_4$ (HAMMARSTEN[4]). The bile of other animals (walrus, seal) contains special cholic acids (HAMMARSTEN[5]).

On boiling with acids, on putrefaction in the intestine, or on heating, cholic acids lose water and are converted into anhydrides, the so-called *dyslysins*. The dyslysin, $C_{24}H_{36}O_3$, corresponding to ordinary cholic acid, which occurs in fæces, is amorphous, insoluble in water and alkalies. *Choloidic acid*, $C_{24}H_{38}O_4$, is called the first anhydride or an intermediary product in the formation of dyslysin. On boiling dyslysins with caustic alkali they are reconverted into the corresponding cholic acids.

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[1] Schotten, Zeitschr. f. physiol. Chem., **11**; Lassar-Cohn, Ber. d. deutsch. chem. Gesellsch., **27**.

[2] See Jünger and Klages, Ber. d. deutsch. chem. Gesellsch. **28** (older literature).

[3] Investigations not published.

[4] Zeitschr. f. physiol. Chem., **36**.

[5] Investigations not published.

The Detection of Bile-acids in Animal Fluids. To obtain the bile-acids pure so that Pettenkofer's test can be applied to them, the protein and fat must first be removed. The protein is removed by making the liquid first neutral and then adding a great excess of alcohol, so that the mixture contains at least 85 vols. per cent of water-free alcohol. Now filter, extract the precipitated protein with fresh alcohol, unite all filtrates, distill the alcohol, and evaporate to dryness. The residue is completely exhausted with strong alcohol, filtered, and the alcohol entirely evaporated from the filtrate. The residue is extracted with ether and dissolved in water, and filtered if necessary, and the solution precipitated by basic lead acetate and ammonia. The washed precipitate is dissolved in boiling alcohol, filtered while warm, and a few drops of soda solution added. Then evaporate to dryness, extract the residue with absolute alcohol, filter, and add an excess of ether. The precipitate now formed may be used for Pettenkofer's test. It is not necessary to wait for crystallization; but one must not consider the crystals which form in the liquid as being positively crystallized bile. It is also possible for needles of alkali acetate to be formed. In this connection it must be remarked that a confusion with phosphatides, which also give Pettenkofer's reaction, is not excluded, and a further testing and separation are advisable.

Bile-pigments. The bile-coloring matters known thus far are relatively numerous, and in all probability there are still more of them. Most of the known bile-pigments are not found in the normal bile, but occur either in post-mortem bile or principally in the bile concrements. The pigments which occur under physiological conditions are the reddish-yellow *bilirubin*, the green *biliverdin*, and sometimes also *urobilin* (and *urobilinogen*) or a closely related pigment. The pigments found in gall-stones are (besides the *bilirubin* and *biliverdin*) *cholepraśin*, *bilifuscin*, *biliprasin*, *bilihumin*, *bilicyanin* (and *choletelin*?). Besides these, others have been noticed in human and animal bile by various observers. The two above-mentioned physiological pigments, bilirubin and biliverdin, are those which serve to give the golden-yellow or orange-yellow or sometimes greenish color to the bile; or when, as is most frequently the case in ox-bile, the two pigments are present in the bile at the same time, they produce the different shades between reddish-brown and green.

Bilirubin. This pigment has the formula $C_{16}H_{18}N_2O_3$, or according to Orndorff and Teeple and Küster,[1] more correctly $C_{32}H_{36}N_4O_6$, and is designated by the names cholepyrrhin, biliphæin, bilifulvin, and hæmatoidin. It occurs chiefly in the gall-stones as calcium bilirubin. Bilirubin is present in the liver-bile of all vertebrates, and in the bladder-bile especially in man and carnivora; sometimes, however, the latter may have a green bile when fasting or in a starving condition. It also occurs in the contents of the small intestine, in the blood serum of the horse,

[1] Orndorff and Teeple, Salkowski's Festschrift, Berlin, 1904; Küster, Zeitschr. f. Physiol. Chem., 59.

in old blood extravasations (as hæmatoidin), and in the urine and the yellow-colored tissue in icterus. It is converted into *hydrobilirubin*, $C_{32}H_{40}N_4O_7$ (Maly), by hydrogen in a nascent state, and then shows great similarity to the urinary pigment, *urobilin*, as well as to *stercobilin* found in the contents of the intestine (Masius and Vanlair[1]). On careful oxidation bilirubin yields biliverdin and other coloring-matters (see below).

Bilirubin is derived from the blood-pigment. It has the same percentage composition as hæmatoporphyrin, and like hæmatin it yields hæmatinic-acid imide as an oxidation product (Küster). On reduction with zinc powder or with nascent HI it yields hæmopyrrol, according to Orndorff and Teeple.[2]

Bilirubin is sometimes amorphous and sometimes crystalline. The amorphous bilirubin is a reddish-yellow or reddish-brown powder; the crystals have a reddish-yellow, reddish-brown, or more reddish color, and sometimes they have nearly the color of crystalline chromic acid. The crystals, which can easily be obtained by allowing a solution of bilirubin in chloroform to evaporate spontaneously, are reddish-yellow, rhombic plates, whose obtuse angles are often rounded. On crystallizing from hot dimethylaniline it forms on cooling broad columns with both ends sharply cut (Küster[3]). On dissolving in chloroform both kinds of crystals are converted into long needles or whetstones.

Bilirubin is insoluble in water, behaves like an acid, and occurs in animal fluids as soluble alkali bilirubin. It is very slightly soluble in ether, benzene, carbon disulphide, amyl alcohol, fatty oils, and glycerin. It is somewhat more soluble in alcohol. In cold chloroform it dissolves with difficulty, and is much more readily soluble in warm chloroform. Its solubility varies, and supersaturated solutions are readily formed (Orndorff and Teeple). The varying solubility of bilirubin in chloroform depends, according to Küster, on the fact that in its preparation derivatives which are readily soluble and contain chlorine or other transformation products are formed, or perhaps the bilirubin goes over into polymeric modifications having different solubilities. In cold dimethylaniline it dissolves in the proportion of 1:100, and in hot dimethylaniline much more readily. Its solutions show no absorption-bands, but only a continuous absorption from the red to the violet end of the spectrum, and they have a decided yellow color, even on diluting greatly (1:500,000), in a layer 1.5 cm. thick. If a dilute solution of

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[1] Maly, Wien. Sitzungsber., **57**, and Annal. d. Chem., **163**; Masius and Vanlair, Centralbl. f. d. med. Wissensch., 1871, 369.

[2] l. c.

[3] Ber. d. d. chem. Gesellsch., **30** and **35**, and Zeitschr. f. physiol. Chem., **47**.

alkali bilirubin in water is treated with an excess of ammonia and then with a zinc-chloride solution, the liquid is first colored deep orange and then gradually olive-brown and then green. This solution first gives a darkening of the violet and blue part of the spectrum, and then the bands of alkaline cholecyanin (see below), or at least the bands of this pigment in the red between *C* and *D*, close to *C*. This is a good reaction for bilirubin. The compounds of bilirubin with alkalies are insoluble in chloroform, and bilirubin may be separated from its solution in chloroform by shaking with dilute caustic alkali (differing from lutein). Solutions of alkali bilirubin in water are precipitated by the soluble salts of the alkaline earths and also by metallic salts.

As EHRLICH first showed, bilirubin forms combinations with diazo compounds, which have been closely studied by PRÖSCHER, ORNDORFF and TEEPLE.[1] A test suggested by EHRLICH for bilirubin is based upon this behavior with diazobenzenesulphonic acid.

If an alkaline solution of bilirubin be allowed to stand in contact with the air, it gradually absorbs oxygen, and green biliverdin is formed. This process is accelerated by warming. According to KÜSTER, in this case the alkali also has a splitting action upon the pigment, and among the products formed we find hæmatinic acid. Biliverdin is formed only from bilirubin by oxidation under special conditions (KÜSTER). A green coloring-matter similar in appearance is formed by the action of other reagents such as Cl, Br, and I. According to JOLLES,[2] biliverdin is produced by the use of HÜBL'S iodine solution, while according to others (THUDICHUM, MALY [3]) substitution products of bilirubin are formed.

GMELIN'S *Reaction for Bile-pigments.* If one carefully pours nitric acid containing some nitrous acid, under an aqueous solution of alkali bilirubin, there is obtained a series of colored layers at the juncture of the two liquids in the following order from above downward: Green, blue, violet, red, and reddish yellow. This color reaction, GMELIN'S test, is very delicate, and serves to detect the presence of one part bilirubin in 80,000 parts liquid. The green ring must never be absent; and also the reddish-violet must be present at the same time, otherwise the reaction may be confused with that for lutein, which gives a blue or greenish ring. The nitric acid must not contain too much nitrous acid, for then the reaction takes place too quickly and it does not become typical. Alcohol must

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[1] Ehrlich, Zeitschr. f. anal. Chem., **23**; Pröscher, Zeitschr. f. physiol. Chem., **39**; Orndorff and Teeple, l. c.

[2] Küster, Ber. d. d. chem. Gesellsch., **35** and **59**; Jolles, Journ. f. prakt. Chem. (N. F.), **59**, and Pflüger's Arch., **75**.

[3] Thudichum, Journ. of Chem. Soc. (2), **13**, and Journ. f. prakt. Chem. (N. F.), **53**; Maly, Wien. Sitzungsber., **72**.

not be present in the liquid, because, as is well known, it gives a play of colors, in green or blue, with the acid.

HAMMARSTEN'S *Reaction.* An acid is first prepared consisting of 1 vol. nitric acid and 19 vols. hydrochloric acid (each acid being about 25-per cent). One volume of this acid mixture, which can be kept for at least a year, is, when it has become yellow by standing, mixed with 4 vols. alcohol. If a drop of bilirubin solution is added to a few cubic centimeters of this colorless mixture a permanent beautiful green color is obtained immediately. On the further addition of the acid mixture to the green liquid all the colors of GMELIN'S scale, as far as choletelin, can be produced consecutively.

HUPPERT'S *Reaction.* If a solution of alkali bilirubin is treated with milk of lime or with calcium chloride and ammonia, a precipitate is produced consisting of calcium bilirubin. If this moist precipitate, which has been washed with water, is placed in a test-tube and the tube half filled with alcohol which has been acidified with hydrochloric acid, and heated to boiling for some time, the liquid becomes emerald-green or bluish green in color.

In regard to the modifications of GMELIN'S test and certain other reactions for bile-pigments, see Chapter XV (Urine).

That the characteristic play of colors in GMELIN'S test is the result of an oxidation is generally admitted. The first oxidation step is the green biliverdin. Then follows a blue coloring matter which HEINSIUS and CAMPBELL call *bilicyanin* and STOKVIS calls *cholecyanin*, and which shows a characteristic absorption-spectrum. The neutral solutions of this coloring-matter are, according to STOKVIS, bluish green or steel-blue with a beautiful blue fluorescence. The alkaline solutions are green and have no marked fluorescence, and show three absorption-bands: one, sharp and dark, in the red between *C* and *D*, nearer to *C*; a second, less well defined, covering *D*; and a third between *E* and *F*, near *E*. The strongly acid solutions are violet-blue and show two bands, described by JAFFÉ, between the lines *C* and *E*, separated from each other by a narrow space near *D*. A third band between *b* and *F* is seen with difficulty. The next oxidation step after these blue coloring-matters is a red pigment, and lastly a yellowish-brown pigment, called *choletelin*, by MALY, which in neutral alcoholic solutions does not give any absorption-spectrum, but in acid solution gives a band between *b* and *F*. On oxidizing cholecyanin with lead peroxide, STOKVIS [1] obtained a product which he calls choletelin, which is quite similar to urinary urobilin, to be discussed later.

[1] Heinsius and Campbell, Pflüger's Arch., 4; Stokvis, Centralbl. f. med. Wissensch., 1872, 785; *ibid.*, 1873, 211 and 449; Jaffé, *ibid.*, 1868; Maly, Wien. Sitzungsber., 59.

Bilirubin is best prepared from gall-stones of oxen, these concretions being very rich in calcium bilirubin. The finely powdered concrement is first exhausted with ether and then with boiling water, so as to remove the cholesterin and bile-acids. In order to remove the mineral constituents it is better to use 10-per cent acetic acid instead of hydrochloric acid (KÜSTER [1]). A green pigment is now removed by extraction with alcohol, and the choleprasin is extracted with hot glacial acetic acid. After washing with water it is dried, and extracted repeatedly with boiling chloroform. The bilirubin separates from the chloroform as crusts, which are treated once or twice in the above manner. It is then extracted with alcohol and precipitated from its chloroform solution by alcohol, or crystallized from boiling dimethylaniline. Further details are given by KÜSTER.[2]

The quantitative estimation of bilirubin may be made by the spectrophotometric method, according to the steps suggested for the blood-coloring matters.

Biliverdin, $C_{16}H_{18}N_2O_4$ or $C_{32}H_{36}N_4O_8$. This body, which is formed by the oxidation of bilirubin, occurs in the bile of many animals, in vomited matter, in the placenta of the bitch (?), in the shells of birds' eggs, in the urine in icterus, and sometimes in gall-stones, although in very small quantities.

Biliverdin is amorphous; at least it has not been obtained in well-defined crystals. It is insoluble in water, ether, and chloroform (this is true at least for the artificially prepared biliverdin), but is soluble in alcohol or glacial acetic acid, showing a beautiful green color. It is dissolved by alkalies, giving a brownish-green color, and this solution is precipitated by acids, as well as by calcium, barium, and lead salts. Biliverdin gives HUPPERT'S, GMELIN'S, and HAMMARSTEN'S reactions, commencing with the blue color. It is converted into hydrobilirubin by nascent hydrogen. On allowing the green bile to stand, also by the action of ammonium sulphide, the biliverdin may be reduced to bilirubin (HAYCRAFT and SCOFIELD [3]).

Biliverdin is most simply prepared by allowing a thin layer of an alkaline solution of bilirubin to stand exposed to the air in a dish until the color is brownish green. The solution is then precipitated by hydrochloric acid, the precipitate washed with water until no HCl reaction is obtained, then dissolved in alcohol and the pigment again separated by the addition of water. Any contaminating bilirubin may be removed by means of chloroform. KÜSTER has shown that the biliverdin is only formed by the oxygen of the air from bilirubin under certain conditions: The presence of 2 molecules caustic alkali with the addition of water so that the solution contains 0.2 per cent and, a temperature not above 5° C.

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[1] Zeitschr. f. physiol. Chem., **47**.

[2] Zeitschr. f. physiol. Chem., **59**.

[3] Centralbl. f. Physiol., **3**, 222, and Zeitschr. f. physiol. Chem., **14**.

Hugounenq and Doyon [1] prepared biliverdin from bilirubin by the action of sodium peroxide and a little hydrochloric acid.

Choleprasin is a green pigment isolated by Küster [2] from gall-stones, which is soluble in glacial acetic acid but insoluble in alcohol. It differs from the other bile-pigments by containing sulphur. On distillation with zinc powder it gives the pyrrol reaction, and on oxidation with chromic acid, Küster could not observe any formation of hæmatinic acid.

Bilifuscin, so named by Städeler,[3] is an amorphous brown pigment soluble in alcohol and alkalies, nearly insoluble in water and ether, and soluble with great difficulty in chloroform (when bilirubin is not present at the same time). Pure bilifuscin does not give Gmelin's reaction. This is also true for the bilifuscin prepared by v. Zumbusch,[4] which is more like a humin substance, and the formula of which is $C_{64}H_{96}N_7O_{14}$. Bilifuscin has been found in gall-stones. *Biliprasin* is a green pigment prepared by Städeler from gall-stones, and is generally considered as a mixture of biliverdin and bilirubin. Dastre and Floresco,[5] on the contrary, consider biliprasin as an intermediate step between bilirubin and biliverdin. According to them it occurs as a physiological pigment in the bladder-bile of several animals, and is derived from bilirubin by oxidation. This oxidation is brought about by an oxidative ferment existing in the bile. *Bilihumin* is the name given by Städeler to that brownish amorphous residue which is left after extracting gall-stones with chloroform, alcohol, and ether. It does not give Gmelin's test. *Bilicyanin* is also found in human gall-stones (Heinsius and Campbell). *Cholohæmatin*, so called by MacMunn, is a pigment often occurring in sheep- and ox-bile and characterized by four absorption-bands, which is formed from hæmatin by the action of sodium amalgam. In the dried condition, as when obtained by the evaporation of the chloroform solution, it is green, and in alcoholic solution olive-brown. This pigment, which has also been found by Hammarsten in the bile from the musk-ox and hippopotamus, is, according to Marchlewski, identical with the crystalline *bilipurpurin* isolated by Loebisch and Fischler from ox-bile. This latter pigment, according to Marchlewski, is not a bile-pigment, but *phylloerythrin*, a transformation product of chlorophyll. Phylloerythrin has been detected by Marchlewski [6] in the excrement of cows fed on green grass.

Gmelin's and Huppert's reactions are generally used to detect the presence of bile-pigments in animal fluids or tissues. The first, as a rule, can be performed directly, and the presence of proteins does not interfere with it, but, on the contrary, it brings out the play of colors more strikingly. If blood-coloring matters are present at the same time, the bile-coloring matters are first precipitated by the addition of sodium phosphate and milk of lime. This precipitate containing the bile-pigments may be used directly in Huppert's reaction, or a little of the precipitate may be dissolved in Hammarsten's reagent. Bilirubin is detected in

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[1] Hugounenq et Doyon, Arch. de Phyisol. (5), 8; Küster, Zeitschr. f. physiol. Chem., **59**.

[2] Zeitschr. f. physiol. Chem., **47**.

[3] Cited from Hoppe-Seyler, Physiol. u. Path. chem. Analyse, 6. Aufl., p. 225.

[4] Zeitschr. f. physiol. Chem., **31**.

[5] Arch. de Physiol. (5), **9**.

[6] MacMunn, Journ. of Physiol., **6**; Loebisch and Fischler, Wien. Sitzungsber., **112** (1903); Marchlewski, Zeitschr. f. physiol. Chem., **41**, **43**, and **45**; Hammarsten, *ibid.*, **43**, and investigations not published.

blood, according to HEDENIUS,[1] by precipitating the proteins with alcohol, filtering and acidifying the filtrate with hydrochloric or sulphuric acid, and boiling. The liquid becomes of a greenish color. Serum and serous fluids may be boiled directly with a little acid after the addition of alcohol.

Besides the bile-acids and the bile-pigments, there occur in the bile also *cholesterin*, *lecithin*, *jecorin* or other *phosphatides* (HAMMARSTEN), *palmitin*, *stearin*, *olein*, *myristic acid* (LASSAR-COHN[2]), *soaps*, *ethereal sulphuric acids*, *conjugated glucuronates*, *diastatic* and *proteolytic enzymes*. *Choline* and *glycerophosphoric acid*, when they are present, may be considered as decomposition products of lecithin. *Urea* occurs, though only in traces, as a physiological constituent of human, ox, and dog bile. Urea occurs in the bile of the shark and ray in such large quantities that it forms one of the chief constituents of the bile.[3] The *mineral constituents* of the bile are, besides the alkalies, to which the bile-acids are united, sodium and potassium chloride, calcium and magnesium phosphate, and iron—0.04–0.115 p. m. in human bile, chiefly combined with phosphoric acid (YOUNG[4]). Traces of copper are habitually present, and traces of zinc are often found. Sulphates are entirely absent, or occur only in very small amounts.

The quantity of iron in the bile varies greatly. According to NOVI it is dependent upon the kind of food, and in dogs it is lowest with a bread diet and highest with a meat diet. According to DASTRE this is not the case. The quantity of iron in the bile varies even though a constant diet is maintained, and the variation is dependent upon the formation and destruction of blood. According to BECCARI[5] the iron does not disappear from the bile in inanition, and the percentage shows no constant diminution. The question as to the extent of elimination by the bile of the iron introduced into the body has received various answers. There is no doubt that the liver has the property of collecting and retaining iron, as well as other metals, from the blood. Certain investigators, such as NOVI and KUNKEL, are of the opinion that the iron introduced and transitorily retained in the liver is eliminated by the bile, while others, such as HAMBURGER, GOTTLIEB, and ANSELM,[6] deny any such elimination of iron by the bile.

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[1] Upsala Läkaref. Förh., **29**, and Maly's Jahresber., **24**.

[2] Zeitschr. f. physiol. Chem., **17**; Hammarsten, *ibid.*, **32**, **36** and **43**.

[3] Hammarsten, *ibid.*, **24**.

[4] Journ. of Anat. and Physiol., **5**, 158.

[6] Novi, see Maly's Jahresber., **20**; Dastre, Arch. de Physiol. (5), **3**; Beccari, Arch. ital. de Biol., **28**.

[5] Kunkel, Pflüger's Arch., **14**; Hamburger, Zeitschr. f. physiol. Chem., **2** and **4**; Gottlieb, *ibid.*, **15**; Anselm, "Ueber die Eisenausscheidung der Galle," Inaug.-Diss. Dorpat, 1891. See also the works cited in footnote **1**, p. 322.

Quantitative Composition of the Bile. Complete analyses of human bile have been made by HOPPE-SEYLER and his pupils. The bile was removed from the gall-bladder of cadavers, hence these analyses can be of little interest. Older and less complete analyses of perfectly fresh human bile have been made by FRERICHS and v. GORUP-BESANEZ.[1] The bile analyzed by them was from perfectly healthy persons who had been executed or accidentally killed. The two analyses of FRERICHS are, respectively, of (I) an 18-year-old and (II) a 22-year-old male. The analyses of v. GORUP-BESANEZ are of (I) a man of 49 and (II) a woman of 29. The results are, as usual, in parts per 1000.

	FRERICHS.		v. GORUP-BESANEZ.	
	I.	II.	I.	II.
Water	860.0	859.2	822.7	898.1
Solids	140.0	140.8	177.3	101.9
Biliary salts	72.2	91.4	107.9	56.5
Mucus and pigments	26.6	29.8	22.1	14.5
Cholesterin	1.6	2.6	47.3	30.9
Fat	3.2	9.2		
Inorganic substances	6.5	7.7	10.8	6.2

Human liver-bile is poorer in solids than the bladder-bile. In several cases it contained only 12–18 p. m. solids, but the bile in these cases is hardly to be considered as normal. JACOBSEN found 22.4–22.8 p. m. solids in a specimen of bile. HAMMARSTEN, who had occasion to analyze the liver-bile in seven cases of biliary fistula, has repeatedly found 25–28 p. m. solids. In a case of a corpulent woman the quantity of solids in the liver-bile varied between 30.10–38.6 p. m. in ten days. BRAND[2] observed still higher figures, more than 40 p. m., in a couple of cases. This investigator suggests that the bile from an imperfect fistula, when it is partly absorbed, is richer in solids than when it comes from a perfect fistula.

The molecular concentration of human bile, according to BRAND, BONANNI, and STRAUSS,[3] is nearly always identical with that of the blood, although the amount of water and solids varies. The freezing-point varies only between −0.54° and −0.58°. This constancy of the osmotic pressure is explained by the fact that in concentrated biles with larger amounts of organic substances (with larger molecules) the amount of inorganic salts is lower.[4]

Human bile sometimes, but not always, contains sulphur in an ethereal

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[1] See Hoppe-Seyler Physiol. Chem., 301; Socoloff, Pflüger's Arch., **12**; Trifanowski, *ibid.*, **9**; Frerichs in Hoppe-Seyler's Physiol. Chem., 299; v. Gorup-Besanez, *ibid.*

[2] Jacobsen, Ber. d. deutsch. chem. Gesellsch., **6**; Hammarsten, Nova Acta Reg. Soc. Scient. Upsala, **16**; Brand, Pflüger's Arch., **90**.

[3] Brand, l. c.; Bonanni, Biochem. Centralbl., **1**; Strauss, Berl. klin. Wochenschr., 1903.

[4] See Brand, l. c.; Hammarsten, l. c.

sulphuric-acid-like combination (HAMMARSTEN, OERUM, BRAND). The quantity of such sulphur may even amount to $\frac{1}{4}$-$\frac{1}{3}$ of the total sulphur. We do not know the nature of these ethereal sulphuric acids. According to OERUM[1] they are not precipitated by lead acetate, but are precipitated by basic lead acetate, especially with ammonia. Human bile is habitually richer in glycocholic than in taurocholic acid. In six cases of liver-bile analyzed by HAMMARSTEN the relation of taurocholic to glycocholic acid varied between 1: 2.07 and 1:14.36. The bile analyzed by JACOBSEN contained no taurocholic acid.

As an example of the composition of human liver-bile the following results of three analyses made by HAMMARSTEN are given. The results are calculated in parts per 1000.[2]

Solids	25.200	35.260	25.400
Water	974.800	964.740	974.600
Mucin and pigments	5.290	4.290	5.150
Bile-salts	9.310	18.240	9.040
Taurocholate	3.034	2.079	2.180
Glycocholate	6.276	16.161	6.860
Fatty acids from soaps	1.230	1.360	1.010
Cholesterin	0.630	1.600	1.500
Lecithin	0.220	0.574	0.650
Fat		0.956	0.610
Soluble salts	8.070	6.760	7.250
Insoluble salts	0.250	0.490	0.210

Among the mineral constituents the chlorine and sodium occur to the greatest extent. The relation between potassium and sodium varies considerably in different samples. Sulphuric acid and phosphoric acid occur only in very small quantities.

BAGINSKY and SOMMERFELD[3] found true mucin, mixed with some nucleoalbumin, in the bladder-bile of children. The bile contained on an average 896.5 p. m. water; 103.5 p. m. solids; 20 p. m. mucin; 9.1 p. m. mineral substances; 25.2 p. m. bile-salts (of which 16.3 p. m. were glycocholate and 8.9 p. m. taurocholate); 3.4 p. m. cholesterin; 6.7 p. m. fat, and 2.8 p. m. leucine.[4]

The quantity of pigment in human bile is, according to NOËL-PATON, 0.4–1.3 p. m. (in a case of biliary fistula). The method used in determining the pigments in this case was not quite trustworthy. More exact results obtained by spectrophotometric methods are on record for dog-bile. According to STADELMANN[5] dog-bile contains on an average

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[1] Skand. Archiv. f. Physiol., **16**.

[2] Recent quantitative analyses may be found in Brand, l. c.; v. Zeynek, Wien. klin. Wochenschr., 1899; Bonanni, l. c.

[3] Verhandl. d. physiol. Gesellsch. zu Berlin, 1894–95.

[4] Analyses of bile from children may be found in Heptner, Maly's Jahresber., **30**.

[5] Noël-Paton, Rep. Lab. Roy. Soc. Coll. Phys. Edinburgh, **3**; Stadelmann, Der Icterus.

0.6–0.7 p. m. bilirubin. At the most only 7 milligrams of pigment are secreted per kilo of body in the twenty-four hours.

In animals the relative proportion of the two acids varies considerably. It has been found, on determining the amount of sulphur, that, so far as the experiments have gone, taurocholic acid is the prevailing acid in carnivorous mammals, birds, snakes, and fishes. Among the herbivora, sheep and goats have a predominance of taurocholic acid in the bile. Ox-bile sometimes contains taurocholic acid in excess, in other cases glycocholic acid predominates, and in a few cases the latter occurs almost alone. The bile of the rabbit, hare, kangaroo, hippopotamus, and orang-utang (HAMMARSTEN [1]) contains, like the bile of the pig, almost exclusively glycocholic acid. A distinct influence on the relative amounts of the two bile-acids exerted by differences in diet has not been detected. RITTER [2] claims to have found a decrease in the quantity of taurocholic acid in calves when they pass from the milk to the vegetable diet.

In the above-mentioned calculation of the taurocholic acid from the quantity of sulphur in the bile-salt, it must be remarked that no exact conclusion can be drawn from such a determination, since it is known that other kinds of bile (e.g., human and shark bile) contain sulphur in compounds other than taurocholic acid.[3]

The phosphorized constituents of bile are not well known; nevertheless, there is no doubt that bile contains other phosphatides besides lecithin (HAMMARSTEN). These phosphatides are in part precipitated in the precipitation of the bile-salts and they in part keep the bile-salts in solution, preventing their complete precipitation, and hence they have a double disturbing action in the quantitative analysis of bile. Those biles richest in phosphatides, so far as known, are the following, in the order of their amount: Polar bear, man (in special cases), dog, black bear, orang-utang. The bile of certain fishes contains but little phosphatides (HAMMARSTEN [4]).

The cholesterin, which, according to several investigators, originates not only from the liver but also from the biliary passages, occurs in larger quantities in the bladder-bile than in the liver-bile, and is present to a greater extent in the non-filtered than in the filtered bile (DOYON and DUFOURT [5]).

The *gases* of the bile consist of a large quantity of carbon dioxide,

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[1] Investigations not published. See Ergebnisse der Physiol., 4.

[2] Cited from Maly's Jahresber., 6, 195.

[3] Hammarsten, Zeitschr. f. physiol. Chem., 32, and Ergebnisse der Physiol., 4.

[4] Zeitschr. f. physiol. Chem., 36, and Ergebnisse der Physiol., 4.

[5] Arch. de Physiol. (5), 8.

which increases with the amount of alkalies, only traces of oxygen, and a very small quantity of nitrogen.

Little is known in regard to the *properties of the bile in disease.* The quantity of *urea* is found to be considerably increased in uræmia. *Leucine* and *tyrosine* are observed in acute yellow atrophy of the liver and in typhoid. Traces of *albumin* (without regard to nucleoalbumin) have several times been found in the human bile. The so-called *pigmentary acholia,* or the secretion of a bile containing bile-acids but no bile-pigments, has also been repeatedly noticed. In all such cases observed by RITTER he found a fatty degeneration of the liver-cells, in return for which, even in excessive fatty infiltration, a normal bile containing pigments was secreted. The secretion of a bile nearly free from bile-acids has been observed by HOPPE-SEYLER [1] in amyloid degeneration of the liver. In animals, dogs, and especially rabbits, it has been observed that the blood-pigments pass into the bile in poisoning and in other conditions, causing a destruction of the blood-corpuscles, as also after intravenous hæmoglobin injection (WERTHEIMER and MEYER, FILEHNE, STERN [2]). Albumin can pass into the bile after the intravenous injection of a foreign protein (casein) (GÜRBER and HALLAUER), as well as after poisoning with phosphorus or arsenic (PILZECKER), or after the irritation of the liver by the introduction of ethyl alcohol or amyl alcohol (BRAUER). Sugar occurs in bile only in exceptional cases.[3]

The physiological secretion of the gall-bladder in man is, according to WAHLGREN,[4] a viscous, alkaline fluid with 11.24–19.63 p. m. solids. The mucilaginous properties are not due to mucin, but to a phosphorized protein substance (nucleoalbumin or nucleoprotein).

Instead of bile there is sometimes found in the gall-bladder under pathological conditions a more or less viscous, thready, colorless fluid which contains pseudo-mucins or other peculiar protein substances.[5]

Chemical Formation of the Bile. The first question to be answered is the following: Do the specific constituents of the bile, the bile-acids and bile pigments originate in the liver; and if this is the case, do they come from this organ alone, or are they also formed elsewhere?

The investigations of the blood, and especially the comparative investigations of the blood of the portal and hepatic veins under normal conditions, have not given any answer to this question. To decide this, therefore, it is necessary to extirpate the liver of animals or to isolate it from the circulation. If the bile constituents are not formed in the liver, or at least not alone in this organ, but are eliminated only from the blood, then, after the extirpation or removal of the liver from the

[1] Ritter, Compt. rend., **74**, and Journ. de l'anat. et de la physiol. (Robin), 1872; Hoppe-Seyler, Physiol. Chem., 317.

[2] Wertheimer and Meyer, Compt. rend., **108**; Filehne, Virchow's Arch., **121**; Stern, *ibid.*, **123**.

[3] Gürber and Hallauer, Zeitschr. f. Biologie, **45**; Pilzecker, Zeitschr. f. physiol. Chem., **41**; Brauer, *ibid.*, **40**.

[4] See Maly's Jahresber., **32**.

[5] Winternitz, Zeitschr. f. physiol. Chem., **21**; Sollmann, Amer. Medicine, **5** (1903).

circulation, an accumulation of the bile constituents is to be expected in the blood and tissues. If the bile constituents, on the contrary, are formed exclusively in the liver, then the above operation naturally would give no such result. If the ductus choledochus is tied, then the bile constituents will be collected in the blood or tissues whether they are formed in the liver or elsewhere.

From these principles KÖBNER has tried to demonstrate by experiments on frogs that the *bile-acids* are produced exclusively in the liver. While he was unable to detect any bile-acids in the blood and tissues of these animals after extirpation of the liver, he was able to discover them on tying the ductus choledochus. The investigations of LUDWIG and FLEISCHL [1] show that in the dog the bile-acids originate in the liver alone. After tying the ductus choledochus they observed that the bile constituents were absorbed by the lymphatic vessels of the liver and passed into the blood through the thoracic duct. Bile-acids could be detected in the blood after such an operation, while they could not be detected in the normal blood. But when the common bile and thoracic ducts were both tied at the same time, then not the least trace of bile-acids could be detected in the blood, while if they are also formed in other organs and tissues they should have been present.

From earlier reports of CLOEZ and VULPIAN, as well as VIRCHOW, the bile-acids also occur in the suprarenal capsule. These claims have not been confirmed by later investigations of STADELMANN and BEIER.[2] At the present time there is no ground for supposing that the bile-acids are formed elsewhere than in the liver.

It has been indubitably proven that the *bile-pigments* may be formed in other organs besides the liver, for, as is generally admitted, the coloring-matter hæmatoidin, which occurs in old blood extravasations, is identical with the bile-pigment bilirubin (see page 290). LATSCHENBERGER [3] also observed in horses, under pathological conditions, a formation of bile-pigments from the blood-coloring matters in the tissues. The occurrence of bile-pigments in the placenta also seems to depend on their formation in that organ, while the occurrence of small quantities of bile-pigments in the blood-serum of certain animals probably depends on an absorption of these substances.

Although the bile-pigments may be formed in other organs besides the liver, still it is of first importance to know what bearing this organ has on the elimination and formation of bile-pigments. In this regard

[1] Köbner, see Heidenhain, Physiologie der Absonderungsvorgänge, in Hermann's Handbuch, 5; Fleischl, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang 9.

[2] Zeitschr. f. physiol. Chem., 18, in which the older literature may be found.

[3] See Maly's Jahresber., 16, and Monatshefte f. Chem., 9.

it must be recalled that the liver is an excretory organ for the bile-pigments circulating in the blood. TARCHANOFF observed, in a dog with biliary fistula, that intravenous injection of bilirubin causes a very considerable increase in the bile-pigments eliminated. This statement has been lately confirmed by the investigations of VOSSIUS.[1]

Numerous experiments have been made to decide the question whether the bile-pigments are only eliminated by the liver or whether they are also formed therein. By experimenting on pigeons, STERN was able to detect bile-pigments in the blood-serum five hours after tying the biliary passages alone, while after tying all the vessels of the liver and also the biliary passages, no bile-pigments could be detected either in the blood or the tissues of the animal, which was killed 10–24 hours after the operation. MINKOWSKI and NAUNYN[2] also found that poisoning with arseniuretted hydrogen produces a liberal formation of bile-pigments, and the secretion, after a short time, of a urine rich in biliverdin in previously healthy geese. In geese with extirpated livers this does not occur.

No such experiments can be carried out on mammalia, as they do not live long enough after the operation; still there is no doubt that this organ is the chief seat of the formation of bile-pigments under physiological conditions.

In regard to the materials from which the bile-acids are produced, it may be said with certainty that the two components, glycocoll and taurine, which are both nitrogenized, are formed from the protein bodies. The close relation of taurine to the cystine group of the protein molecule has been especially shown by the investigations of FRIEDMANN (see Chapter III), and recently v. BERGMANN[3] has shown by feeding dogs with sodium cholate and cystine that the animal body can transform cystine into taurine and that the taurine of the bile originates from the proteins of the food. In regard to the origin of the non-nitrogenized cholic acid, which was formerly considered as originating from the fats, nothing is positively known.

The blood-coloring matters are considered as the mother-substances of the bile-pigments. If the identity of hæmatoidin and bilirubin was settled beyond a doubt, then this view might be considered as proven. Independently, however, of this identity, which is not admitted by all investigators, the view that the bile-pigments are derived from the blood-coloring matters has strong arguments in its favor. It has been shown by several experimenters that a yellow or yellowish-red pigment

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[1] Tarchanoff, Pflüger's Arch., **9**; Vossius, cited from Stadelmann, Der Icterus.

[2] Stern, Arch. f. exp. Path. u. Pharm., **19**; Minkowski and Naunyn, *ibid.*, **21**.

[3] Hofmeister's Beiträge, **4**. See also Wohlgemuth, Zeitschr. f. physiol. Chem., **40**.

can be formed from the blood-coloring matters, which gives GMELIN'S test, and which, though it may not form a complete bile-pigment, is at least a step in its formation (LATSCHENBERGER). A further proof of the formation of the bile-pigments from the blood-coloring matters consists in the fact that hæmatin on reduction yields urobilin, which is identical with hydrobilirubin (see Chapter XV). Further, hæmatoporphyrin (see page 288) and bilirubin are isomers, according to NENCKI and SIEBER, and closely allied. The formation of bilirubin from the blood-coloring matters is shown, according to the observations of several investigators,[1] by the fact that the appearance of free hæmoglobin in the plasma, produced by the destruction of the red corpuscles by widely differing influences (see below) or by the injection of hæmoglobin solution, causes an increased formation of bile-pigments. The amount of pigments in the bile is not only considerably increased, but the bile-pigments may even pass into the urine under certain circumstances (icterus). After the injection of hæmoglobin solution into a dog either subcutaneously or in the peritoneal cavity, STADELMANN and GORODECKI[2] observed an increase of 61 per cent in the secretion of pigments by the bile, which lasted for more than twenty-four hours.

If bilirubin, which contains no iron, is derived from hæmatin, which contains iron, then iron must be split off. This process may be represented by the following equation: $C_{32}H_{34}N_4O_5Fe + H_2O - Fe = C_{32}H_{36}N_4O_6$. The question in what form or combination the iron is split off is of special interest, and also whether it is eliminated by the bile. This latter does not seem to be the case, at least to any great extent. In 100 parts of bilirubin which are eliminated by the bile there are only 1.4–1.5 parts iron, according to KUNKEL, while 100 parts hæmatin contain about 9 parts iron. MINKOWSKI and BASERIN[3] also found that the abundant formation of bile-pigments occurring in poisoning by arseniuretted hydrogen does not increase the quantity of iron in the bile. The quantity apparently does not seem to correspond with that in the decomposed blood-coloring matters. It follows from the researches of several investigators[4] that the iron is, at least chiefly, retained by the liver as a ferruginous pigment or protein substance.

What relation does the formation of bile-acids bear to the formation of bile-pigments? Are these two chief constituents of the bile derived simultaneously from the same material, and can we detect a certain

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[1] See Stadelmann, Der Icterus, etc., Stuttgart, 1891.

[2] See Stadelmann, *ibid.*

[3] Kunkel, Pflüger's Arch., **14**; Minkowski and Baserin, Arch. f. exp. Path. u. Pharm., **23**.

[4] See Naunyn and Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Latschenberger, l.c.; Neumann, Virchow's Arch., **111**, and the literature in footnote 2, p. 362.

connection between the formation of bilirubin and bile-acids in the liver? The investigations of STADELMANN teach us that this is not the case. With increased formation of bile-pigments the amount of bile-acids is decreased, and the introduction of hæmoglobin into the liver strongly increases the formation of bilirubin, but simultaneously strongly decreases the production of bile-acids. According to STADELMANN the formation of bile-pigments and bile-acids is due to a special activity of the cells.

An absorption of bile from the liver and the passage of the bile constituents into the blood and urine occurs in retarded discharge of the bile, and usually in different forms of *hepatogenic icterus*. But bile-pigments may also pass into the urine under other circumstances, especially when a solution or destruction of the red blood-corpuscles takes place in animals through injection of water or a solution of biliary salts, through poisoning by ether, chloroform, arseniuretted hydrogen, phosphorus, or toluylenediamine, and in other cases. This also occurs in man in severe infectious diseases. It has also been claimed many times that a transformation of blood-pigments into bile-pigments occurs elsewhere than in the liver, namely, in the blood. Such a belief has been made very improbable by the important researches of MINKOWSKI and NAUNYN, AFANASSIEW, SILBERMANN, and especially of STADELMANN,[1] and in some of the above-mentioned cases, as after poisoning with phosphorus, toluylenediamine, and arseniuretted hydrogen, it has been disproven by direct experiment.

The icterus is also in these cases hepatogenic; it depends upon an absorption of bile-pigments from the liver, and this absorption seems to originate in various cases in somewhat different ways. Thus the bile may be viscous and cause a congestion of bile by counteracting the low secretion pressure. In other cases the fine biliary passages may be compressed by an abnormal swelling of the liver-cells, or a catarrh of the bile-passages may occur, causing a congestion of the bile (STADELMANN).

Bile Concretions.

The concrements which occur in the gall-bladder vary considerably in size, form, and number, and are of three kinds, depending upon the kind and nature of the bodies forming their chief mass. One group of gallstones contains lime-pigment as chief constituent, another cholesterin, and the third calcium carbonate and phosphate. The concrements of the last-mentioned group occur very seldom in man. The so-called cholesterin-stones are those which occur most frequently in man, while

[1] The literature belonging to this subject is found in Stadelmann, Der Icterus, etc., Stuttgart, 1891.

the lime-pigment stones are not found very often in man, but often in oxen.

The *pigment-stones* are generally not large in man, but in oxen and pigs they are sometimes found the size of a walnut or even larger. In most cases they consist chiefly of calcium-bilirubin with little or no biliverdin. Sometimes also small black or greenish-black, metallic-looking stones are found, which consist chiefly of bilifuscin along with biliverdin. Iron and copper seem to be regular constituents of pigment-stones. Manganese and zinc have also been found in a few cases. The pigment-stones are generally heavier than water.

The *cholesterin-stones*, whose size, form, color, and structure may vary greatly, are often lighter than water. The fractured surface is radiated, crystalline, and frequently shows crystalline, concentric layers. The cleavage fracture is waxy in appearance, and the fractured surface when rubbed by the finger-nail also becomes like wax. By rubbing against each other in the gall-bladder they often become faceted or take other remarkable shapes. Their surface is sometimes nearly white and wax-like, but generally their color is variable. They are sometimes smooth, in other cases they are rough or uneven. The quantity of cholesterin in the stones varies from 642 to 981 p. m. (RITTER [1]). The cholesterin-stones sometimes contain variable amounts of lime-pigments, which may give them a very changeable appearance.

Cholesterin. The formula for this body, although not positively determined, is generally given as $C_{27}H_{46}O$ (OBERMÜLLER) or $C_{27}H_{44}O$ (MAUTHNER and SUIDA).

Because of the fact that from cholesterin, hydrocarbons which have been called *cholesteriline*, *cholesterone* and *cholesterilene*, can be prepared in different ways, it was believed that a certain analogy exists between the cholesterin and the terpenes. The color reactions as well as the recent investigations on the constitution of cholesterin indicate that this body belongs to the terpenes.

The constitution of cholesterin has not been completely determined, although we have the very laborious and thorough investigations, of many workers of whom we especially mention MAUTHNER and SUIDA, WINDAUS, STEIN, DIELS and ABDERHALDEN.[2] From these investigations we conclude that cholesterin is a monoatomic, unsaturated, secondary alcohol whose hydroxyl group exists in a hydrogenized ring, indeed between two methyl groups, and which also contains an isopropyl group. It is also generally admitted that cholesterin contains only one double

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[1] Journ. de l'anat. et de la physiol. (Robin), 1872.

[2] The literature on cholesterin can be found in Windaus, Arch. d. Pharm., 246, Hft. 2, and especially in Glikin, Bioch. Centralbl., 7, 372–377.

bond, which occurs in a vinyl group $CH:CH_2$ at the end. Contrary to this MOLINARI and FENAROLI,[1] basing their opinions upon the behavior of cholesterin with ozone, believe the cholesterin has two double bonds. Under these circumstances no constitutional formula for cholesterin can be given; still there is no doubt that it is a complex terpene which stands in close relation to retene as well as to the cholic acids.

By the reduction of cholesterin by metallic sodium and amyl alcohol, DIELS and ABDERHALDEN as well as NEUBERG and RAUCHWERGER obtained a dihydrocholesterin, the *α-cholestanol*, $C_{27}H_{48}O$. On treating cholestenon, the ketone of cholesterin, DIELS and ABDERHALDEN obtained a second dihydrocholesterin, the *β-cholestanol*, which WILLSTÄTTER and E. W. MAYER obtained directly from cholesterin in ethereal solution by reduction with hydrogen and platinum-black. According to DIELS and LINN[2] β-cholestanol is obtained from cholestenon by the action of sodium and amyl alcohol, and α-cholestanol with sodium amylate. The relation of these bodies to each other is still not understood. These dihydrocholesterins have a physiological interest in regard to the question whether they are identical or not with koprosterin, which will be discussed below. For the present this question must be answered in the negative.

On heating cholesterin, when contaminated with iron, to 300–320°, according to DIELS and LINN,[3] it in part yields cholestenon and partly an isomeric cholesterin, the *β-cholesterin*. This last body can be retransformed into cholesterin by the saponification of the cholesteryl benzoate.

Cholesterin occurs in small amounts in nearly all animal fluids and juices. It occurs only rarely in the urine, and then in very small quantities. It is also found in the different tissues and organs, especially abundant in the brain and the nervous system; further, in the yolk of the egg, in semen, in wool-fat (together with isocholesterin), and in sebum. It also appears in the contents of the intestine, in excrements, and in the meconium. It especially occurs pathologically in gall-stones as well as in atheromatous cysts, in pus, in tuberculous masses, old transudates, cystic fluids, sputum, and tumors. It does not exist free in all cases; for example, it exists in part as fatty-acid esters in wool-fat, blood, lymph, brain vernix caseosa, and epidermis formations. Several kinds of cholesterin, called *phytosterines*, have been found in the vegetable kingdom.

Cholesterin which has been crystallized from warm alcohol on cooling, and also that which is present in old transudates, contains one molecule of water of crystallization, and melts at 148.5° C. when dried in a vacuum, and forms colorless, transparent plates whose sides and angles frequently appear broken, and whose acute angle is often 76° 30′ or 87° 30′. In large quantities it appears as a mass of white plates which shine like mother-of-pearl and have a greasy touch.

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[1] Ber. d. d. chem. Gesellsch., **41**.

[2] Willstatter and Mayer, Ber. d. d. chem. Gesellsch., **41**; Diels and Linn, *ibid.*, **41**.

[3] *Ibid.*, **41**.

Cholesterin is insoluble in water, dilute acids, and alkalies. It is neither dissolved nor changed by boiling caustic alkali. It is easily soluble in boiling alcohol, and crystallizes on cooling. It dissolves readily in ether, chloroform, and benzene, and also in the volatile or fatty oils. It is dissolved to a slight extent by alkali salts of the bile-acids, better in the presence of oleic soap (GERARD [1]). The solutions in ether and chloroform are levorotatory, $(\alpha)_D = -31.12°$ (2-per cent ethereal solution).

Among the many compounds of cholesterin the propionic ester $C_2H_5.CO.O.C_{27}H_{45}$ is of special interest because of the behavior of the fused compound on cooling, and it is used in the detection of cholesterin. For the detection of cholesterin use is made of its reaction with concentrated sulphuric acid, which gives colored products.

If a mixture of five parts sulphuric acid and one part water acts on cholesterin crystals, they show colored rings, first a bright carmine-red and then violet. This fact is employed in the microscopic detection of cholesterin. Another test, and one very good for the microscopical detection of cholesterin, consists in treating the crystals first with the above dilute acid and then with some iodine solution. The crystals will be gradually colored violet, bluish green, and a beautiful blue.

SALKOWSKI'S [2] *Reaction.* The cholesterin is dissolved in chloroform and then treated with an equal volume of concentrated sulphuric acid. The cholesterin solution becomes first bluish red, then gradually more violet-red, while the sulphuric acid appears dark red with a greenish fluorescence. If the chloroform solution is poured into a porcelain dish it becomes violet, then green, and finally yellow.

LIEBERMANN-BURCHARD'S [3] *Reaction.* Dissolve the cholesterin in about 2 cc. chloroform and add first 10 drops of acetic anhydride and then concentrated sulphuric acid drop by drop. The color of the mixture will first be a beautiful red, then blue, and finally, if not too much cholesterin or sulphuric acid is present, a permanent green. In the presence of very little cholesterin the green color may appear immediately.

NEUBERG-RAUCHWERGER'S [4] *Reaction.* With rhamnose, or better still with δ-methylfurfurol and concentrated sulphuric acid, an alcoholic solution of cholesterin gives a pink ring, or after mixing the liquids and cooling, a pink solution. On proper dilution an absorption-band can be seen just beginning before *E* and whose other side coincides with *b*. This reaction is of interest because it is also given by bile-acids, some

[1] Compt. rend. soc. biol., 58.

[2] Pflüger's Arch., 6.

[3] C. Liebermann, Ber. d. deutsch. chem. Gesellsch., 18; 1804, H. Burchard, Beiträge zur Kenntnis der Cholesterine, Rostock, 1899.

[4] Salkowski's Festschrift, 1904.

camphor derivatives, abietinic acid, and a hydride of retene. For details of its performance, see original publication.

LIFSCHÜTZ'S *Reaction.*[1] Dissolve a few milligrams of cholesterin in 2–3 cc. glacial acetic acid, add a little benzoylsuperoxide thereto, and boil once or twice. On adding 4 drops concentrated sulphuric acid to the cooled solution and shaking, a pure green coloration is obtained, which changes immediately into blue or with violet-red as an intermediary color. An absorption-band is formed between *C* and *d* and a broad band at *D*. In this reaction an oxidation of the cholesterin occurs, and LIFSCHÜTZ[2] therefore uses the glacial acetic acid-sulphuric acid reaction (color and spectrum) for the detection of oxidation products of cholesterin in blood and tissues.

Pure, dry cholesterin when fused in a test-tube over a low flame with two or three drops of propionic anhydride yields a mass which on cooling is first violet, then blue, green, orange, carmine-red, and finally copper-red. It is best to re-fuse the mass on a glass rod and then to observe the rod on cooling, holding it against a dark background (OBERMÜLLER[3]).

SCHIFF'S *Reaction.* If a little cholesterin is placed in a porcelain dish with the addition of a few drops of a mixture of 2 or 3 vols. of concentrated hydrochloric acid or sulphuric acid and 1 vol. of a rather dilute solution of ferric chloride, and carefully evaporated to dryness over a small flame, a reddish-violet residue is first obtained and then a bluish-violet.

If a small quantity of cholesterin is evaporated to dryness with a drop of concentrated nitric acid, one obtains a yellow spot which becomes deep orange-red with ammonia or caustic soda (not a characteristic reaction).

Koprosterin is the name given by BONDZYNSKI to the cholesterin which was isolated by him from human feces, although it was prepared earlier by FLINT and designated as *stercorin.* It dissolves in cold, absolute alcohol and very readily in ether, chloroform, and benzene. It crystallizes in fine needles which melt at 95–96° C. (89–90° according to HAUSMANN), and is dextrorotatory, $(\alpha)_D = +24°$. It gives the same color reactions as cholesterin, with the exception that it does not give a reaction with propionic anhydride. According to BONDZYNSKI and HUMNICKI it is a dihydrocholesterin, with the formula $C_{27}H_{48}O$, which is formed in the human intestine by the reduction of ordinary cholesterin. According to KUSUMOTO as well as DORÉE and GARDNER,[4] koprosterin also occurs in the intestine of dogs.

Hippokoprosterin is another cholesterin richer in hydrogen, which BONDZYNSKI and HUMNICKI found in the feces of the horse. Its formula is $C_{27}H_{54}O$. According to DORÉE and GARDNER it is not an animal cleavage product, but a constituent of the grass used as fodder. It melts at 78.5–79.5° C.

Isocholesterin is a cholesterin, so called by SCHULZE,[5] with the formula

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[1] Ber. d. d. chem. Gesellsch., **41**.

[2] Zeitschr. f. physiol. Chem., **50**, **53**, **58**, and Ber. d. d. chem. Gesellsch., **41**.

[3] Zeitschr. f. physiol. Chem., **15**.

[4] Bondzynski, Ber. d. deutsch. chem. Gesellsch., **29**; Bondzynski and Humnicki, Zeitschr. f. physiol. Chem., **22**; Flint, *ibid.*, **23**, and Amer. Journ. Med. Sciences, 1862; Müller, Zeitschr. f. physiol. Chem., **29**; Hausmann, Hofmeister's Beiträge, **6**; Kusumoto, Bioch. Zeitschr., **14**; Dorée and Gardner, Proc. Roy. Soc. London, **80**, Ser. B.

[5] Ber. d. deutsch. chem. Gesellsch., **6**; Journal f. prakt. Chem. (N. F.), **25**; and Zeitschr. f. physiol. Chem., **14**, 522. See also E. Schulze and J. Barbieri, Journal f.

$C_{26}H_{43}OH$, which occurs in wool-fat, and is therefore found to a great extent in so-called lanolin. It gives the LIEBERMANN-BURCHARD reaction, but does not give SALKOWSKI's reaction. It melts at 138–138.5° C. The specific rotation in 7-per cent ethereal solution is $(\alpha)_D = +60°$.

Spongosterin is the name given by HENZE [1] to a cholesterin isolated by him from a silicious sponge. It is very similar to cholesterin, but is not identical with it or with phytocholesterins. It gives the LIEBERMANN-BURCHARD reaction as well as SALKOWSKI's reaction, but the last test is not quite so beautiful a red. OBERMÜLLER's reaction is negative. Melting-point 123–124°.

Bombicesterin is the name given by MENOZZI and MORESCHI [2] to a cholesterin isolated by them from the chrysalis of the silkworm, which has a melting-point of 148° and a specific rotation of $(\alpha)_D = -34°$.

The cholesterin occurring in the intestine is derived in part from the food, in part from the bile and part, as shown from the contents of a ligatured portion of the intestine (see Chapter IX), from the epithelium or the secretion of the intestinal mucosa. That a part of the cholesterin of the intestine disappears has been shown by KUSUMOTO, although it remains undecided whether this takes place by bacterial decomposition or by absorption. LEVITES [3] on the contrary, recovered the cholesterin introduced into dogs almost quantitatively. The behavior of cholesterin in metabolism is not well known; LIFSCHÜTZ believes that he has detected by his color-reaction the oxidation products of cholesterin in the blood and in bone-fat.

The cholesterins belong to the so-called lipoids, which have been mentioned in previous chapters (I and VI), and are of the greatest importance as constituents of the outer envelope of erythrocytes and the cells in general. Cholesterin is also of great interest because it inhibits or prevents the hæmolysis produced by certain bodies, and therefore acts as a certain protective power in the animal body. This action of the cholesterins in regard to inhibiting the hæmolytic action of saponin, as first discovered by RANSOM, is destroyed, as shown by HAUSMANN, by replacing the hydroxyl groups. These combinations between cholesterin and saponins have been studied by MADSEN and NOGUCHI and by WINDAUS.[4]

The so-called cholesterin-stones are best employed in the preparation of cholesterin. The powder is first boiled with water and then repeatedly boiled with alcohol. The cholesterin which on cooling separates from the warm filtered solution is boiled with a solution of caustic potash in alcohol

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prakt. Chem. (N. F.), **25**, 159. In regard to the formula for isocholesterin, see Darmstädter and Lifschütz, Ber. d. deutsch. chem. Gesellsch., **31**, and E. Schulze, *ibid.*, 1200.

[1] Zeitschr. f. physiol. Chem., **41** and **55**.

[2] Cited from Chem. Centralbl., 1908.

[3] Zeitschr. f. physiol. Chem., **57**.

[4] Ransom, Deutsch. med. Wochenschr., 1901; Hausmann, Hofmeister's Beiträge, **6**; Madsen and Noguchi, Kgl. Dansk. Vidensk. Selskabs. Forh., 1904; Windaus, Ber. d. d. chem. Gesellsch., **42**.

so as to saponify any fat. After the evaporation of the alcohol the cholesterin is extracted from the residue with ether, by which the soaps are not dissolved; filter, evaporate the ether, and purify the cholesterin by recrystallization from alcohol-ether. The cholesterin may be extracted with fat from tissues and fluids by first extracting with ether and then proceeding as suggested by RITTER.[1] The essential points in his method consist in saponifying the fat with sodium alcoholate, removing the alcohol by evaporating to dryness with NaCl, and finally extracting the dried, pulverized mass with ether. After evaporating the ether the residue is dissolved in as little alcohol as possible and the cholesterin precipitated by the addition of water. It is ordinarily easily detected in transudates and pathological formations by means of the microscope.

[1] Zeitschr. f. physiol. Chem., **34.**

CHAPTER IX.

DIGESTION.

The purpose of digestion is to separate those constituents of the food which serve as nutriment for the body from those which are useless, and to separate each in such a form that it may be taken up by the blood from the alimentary canal and employed for various purposes in the organism. This demands not only mechanical, but also chemical, action. The first action, which is essentially dependent upon the physical properties of the food, consists in a tearing, cutting, crushing, or grinding of the food, while the second serves chiefly in converting the nutritive bodies into a soluble and easily absorbable form, or in splitting them into simpler compounds for use in the animal syntheses. The solution of the nutritive bodies may take place in certain cases by the aid of water alone, but in most cases a chemical metamorphosis or cleavage is necessary; this is effected by means of the acid or alkaline fluids secreted by the glands. The study of the processes of digestion from a chemical standpoint must therefore begin with the digestive fluids, their qualitative and quantitative composition, as well as their action on the nutriments and foods.

I. THE SALIVARY GLANDS AND THE SALIVA.

The **salivary glands** are partly *albuminous glands* (as the parotid in man and mammals and the submaxillary in rabbits), partly *mucous glands* (as some of the small glands in the buccal cavity and the sublingual and sub-maxillary glands of many animals), and partly *mixed glands* (as the submaxillary gland in man). The alveoli of the albuminous glands contain cells which are rich in protein but which contain no mucin. The alveoli of the mucin-glands contain cells rich in mucin but poor in protein. Cells arranged in different ways, but rich in proteins, also occur in the submaxillary and sublingual glands. According to the analyses of Oidtmann [1] the salivary glands of a dog contain 790 p. m. water, 200 p. m. organic and 10 p. m. inorganic solids. Among the solids we find *mucin, proteins, nucleoproteins, nuclein, enzymes* and their *zymogens,* besides *extractive bodies, leucine, purine bases,* and *mineral substances.*

[1] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 732. The figures there given amount to 1010 parts instead of 1000 parts.

The occurrence of a mucinogen has not been proven. On the complete removal of all mucin E. Holmgren [1] found no mucinogen in the submaxillary gland of the ox, but a mucin-like gluconucleoproteid.

The **saliva** is a mixture of the secretion of the above-mentioned groups of glands; therefore it is proper that a study be made of each of the different secretions by itself and then of the mixed saliva.

The **submaxillary saliva** in man may be easily collected by introducing a canula through the papillary opening into Wharton's duct.

The submaxillary saliva has not always the same composition or properties; this depends essentially, as shown by experiments on animals, upon the conditions under which the secretion takes place. That is to say, the secretion is partly dependent on the cerebral system, through the facial fibers in the chorda tympani, and partly on the sympathetic nervous system, through the fibers entering the vessels in the gland. In consequence of this dependence the two distinct varieties of submaxillary secretion are distinguished as *chorda-* and *sympathetic* saliva. A third kind of saliva, the so-called *paralytic saliva*, is secreted after poisoning with curare or after the severing of the glandular nerves.

The difference between chorda- and sympathetic saliva (in dogs) consists chiefly in their quantitative constitution; the less abundant sympathetic saliva is more viscous and richer in solids, especially in mucin, than the more abundant chorda-saliva. The specific gravity of the chorda-saliva of the dog is 1.0039–1.0056, and contains 12–14 p. m. solids (Eckhard [2]). The sympathetic has a specific gravity of 1.0075–1.018, with 16–28 p. m. solids. The freezing-point of the chorda-saliva obtained from dogs on electric stimulation varies, according to Nolf,[3] between $\Delta = -0.193°$ and $-0.396°$, with a content of 3.3–6.5 p. m. salts and 4.1–11.5 p. m. organic substances. The osmotic pressure is on an average a little higher than one-half the osmotic pressure of the blood-serum. The spontaneously secreted submaxillary saliva is ordinarily somewhat diluted. Other investigators, such as Asher and Cutter, and Jappelli [4] also found that the osmotic pressure of the submaxillary saliva is considerably lower than that of the blood. On changing the osmotic pressure of the blood the osmotic pressure of the saliva, according to Jappelli, changes in the same direction; the difference between the pressure of both fluids remains constant. The gases of the chorda-saliva have been investigated by Pflüger.[5] He found 0.5–0.8 per cent oxygen, 0.9–1 per cent nitrogen, and 64.73–85.13 per cent carbon

[1] Upsala Läkaref. Förh. (N. F.), 2; also Maly's Jahresber., 27.

[2] Cited from Kühne's Lehrb. d. physiol. Chem., 7.

[3] See Maly's Jahresber., 31, 494.

[4] Asher and Cutter, Zeitschr. f. Biol., 40; Jappelli, *ibid.*, 48 and 51.

[5] Pflüger's Arch., 1.

dioxide—all results calculated at 0° C. and 760 mm. pressure. The greater part of the carbon dioxide was chemically combined.

The two kinds of submaxillary secretion just named have not thus far been separately studied in man. The secretion may be excited by an emotion, by mastication, and by irritating the mucous membrane of the mouth, especially with acid-tasting substances. The submaxillary saliva in man is ordinarily clear, rather thin, a little ropy, and froths easily. Its reaction is alkaline toward litmus. The specific gravity is 1.002–1.003, and it contains 3.6–4.5 p. m. solids.[1] As organic constituents are found mucin, traces of protein and diastatic enzyme, which latter is absent in several species of animals. The inorganic bodies are alkali chlorides, sodium and magnesium phosphates, and bicarbonates of the alkalies and calcium. Potassium sulphocyanide occurs in this saliva.

The Sublingual Saliva. The secretion of this saliva is also influenced by the cerebral and the sympathetic nervous system. The chorda-saliva, which is secreted only to a small extent, contains numerous salivary corpuscles, but is otherwise transparent and very ropy. Its reaction is alkaline, and it contains, according to HEIDENHAIN,[2] 27.5 p. m. solids (in dogs).

The sublingual secretion in man is clear, mucilaginous, more alkaline than the submaxillary saliva, and contains mucin, diastatic enzyme, and potassium sulphocyanide.

Buccal mucus can be obtained pure from animals only by the method suggested by BIDDER and SCHMIDT, which consists in tying the exit to all the large salivary glands and cutting off their secretion from the mouth. The quantity of liquid secreted under these circumstances (in dogs) was so very small that the investigators named were able to collect only 2 grams of buccal mucus in the course of one hour. It is a thick, ropy, sticky liquid containing mucin; it is rich in form-elements, above all in flat epithelium cells, mucous cells, and salivary corpuscles. The quantity of solids in the buccal mucus of the dog is, according to BIDDER and SCHMIDT,[3] 9.98 p. m.

Parotid Saliva. The secretion of this saliva is also partly dependent on the cerebral nervous system (n. glossopharyngeus) and partly on the sympathetic. The secretion may be excited by emotions and by irritation of the glandular nerves, either directly (in animals) or reflexly, by mechanical or chemical irritation of the mucous membrane of the mouth. Among the chemical irritants the acids take first place. Mas-

[1] See Maly's "Chemie der Verdauungssäfte und der Verdauung," in Hermann's Handb., 5, part II, 18. This article contains also the pertinent literature.

[2] Studien d. physiol. Instituts zu Breslau, Heft 4.

[3] Die Verdauungssäfte und der Stoffwechsel (Mitau and Leipzig, 1852), p. 5.

tication also exercises a strong influence upon the secretion of parotid saliva, which is specially marked in certain herbivora.

Human parotid saliva may be readily collected by the introduction of a canula into STENSON'S duct. This saliva is thin, less alkaline than the submaxillary saliva (the first drops are sometimes neutral or acid), without special odor or taste. It contains a little protein but no mucin, which is to be expected from the construction of the gland. It also contains a diastatic enzyme, which, however, is absent in many animals. The quantity of solids varies between 5 and 16 p. m. The specific gravity is 1.003–1.012. Potassium sulphocyanide seems to be present, though it is not a constant constituent. KÜLZ [1] found a maximum of 1.46 per cent oxygen, 3.8 per cent nitrogen, and in all 66.7 per cent carbon dioxide in human parotid saliva. The quantity of firmly combined carbon dioxide was 62 per cent.

The quantity and composition of the saliva from the mucin glands as well as from the albuminous glands, as PAWLOW'S [2] school has shown, is greatly dependent in dogs upon the psychical excitement, but also upon the kind of substances introduced into the mouth, and an adaptation of the glands for various mechanical and chemical irritants is found to occur. Under the influence of hard and dry food the glands secrete an abundance of saliva, while with food rich in water the secretion is considerably less and accommodates itself to the quantity of water in the food. Milk is an exception to this rule, as it causes a more abundant secretion of saliva than meat. This is of importance in digestion of milk, as in the stomach the mixture of milk and saliva does not coagulate to a compact mass, but separates in a finely divided, readily digestible condition. By the action of strong chemical bodies the saliva is secreted in proportion to the strength of the irritant. According to POPIELSKI [3] this is true only for irritants of medium strength, as after stronger irritation, for example with capsicin solution, the quantity of saliva decreases with the increase in the amount of irritant. The irritating substance is diluted by the saliva and the mouth is washed out at the same time (PAWLOW). The partaking of acid produces, according to PAWLOW, a thin saliva, poor in mucin, in quantities sufficient to neutralize the acid. This claim does not agree with the observations of POPIELSKI, who found that isomolecular acid solutions produced the secretion of the same amount of saliva, and that on using isopercentage acids the quantity of saliva was in inverse proportion to the molecular weight of the acid.

[1] Zeitschr. f. Biologie, **23**.

[2] Arch. internat. de Physiol., **1**, 1904. See also Boos, Maly's Jahresber., **36**, 390, and Neilson and Terry, Amer. Journ. of Physiol., **15**, as well as the work of Mendel and Underhill, Journ. of biol. Chem., **3**.

[3] Pflüger's Arch., **127**.

Popielski also disputes the assumption of Pawlow that the secretion of saliva (in dogs) accommodates itself to the kind of irritant and to the kind of food; although the theory that in man there is an accommodation of the secretion to circumstances has found supporters, yet belief in it is not general.[1]

The mixed buccal saliva in man is a colorless, faintly opalescent, slightly ropy, easily frothing liquid without special odor or taste. It is made turbid by epithelium cells, mucous and salivary corpuscles, and often by food residues. Like the submaxillary and parotid saliva, on exposure to the air it becomes covered with an incrustation consisting of calcium carbonate and a small quantity of an organic substance, or it gradually becomes cloudy. Its reaction is generally alkaline to litmus. The degree of alkalinity varies considerably not only in different individuals but also in the same individual during different parts of the day, so that it is difficult to state the average alkalinity. According to Chittenden and Ely it corresponds to the alkalinity of 0.8 p. m. Na_2CO_3 solution, or to 0.2 p. m. solution according to Cohn. According to Foa the the actual alkalinity (OH-ion concentration) is always considerably less than that found by titration, and the reaction determined electrometrically is very nearly neutral. The reaction may also be acid, as found to be the case by Sticker some time after a meal, but this is not true, at least for all individuals. The specific gravity varies between 1.002 and 1.008, and the quantity of solids between 5 and 10 p. m. According to Cohn,[2] $\Delta = -0.20°$ on an average, and the amount of NaCl is 1.6 p. m. The solids, irrespective of the form-constituents mentioned, consist of *protein, mucin, oxidases,*[3] two enzymes, *ptyalin* and *maltase,* and *mineral bodies.* It is also claimed that *urea* is a normal constituent of the saliva. The mineral bodies are alkali chlorides, bicarbonates of the alkalies and calcium, phosphates, and traces of sulphates, nitrites, ammonia, and sulphocyanides, which latter average about 0.1 p. m. (Munk and others). Smaller quantities, 0.03–0.04 p. m., are found in the saliva of non-smokers (Schneider and Krüger), while from ordinary smokers the quantity of sulphocyanides may rise to 0.2 p. m. (Fleckseder[4]).

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[1] See Zebrowski, Pflüger's Arch., **110**; Neilson and Lewis, Journ. of biol. Chem., **4**, and with Scheele, *ibid.*, **5**.

[2] Chittenden and Ely, Amer. Chem. Journ., **4**, 1883; Chittenden and Richards, Amer. Journ. of Physiol., **1**; Foa, Compt. rend. soc. biol., **58**; Sticker, cited from Centralbl. f. Physiol., **3**, 237; Cohn, Deutsch. med. Wochenschr., 1900.

[3] Bogdanow-Beresowski, cited from Biochem. Centralbl., **2**, 653.

[4] Munk, Virchow's Arch., **69**; Schneider, Amer. Journ. of Physiol., **5**; Krüger, Zeitschr. f. Biologie, **37**; Fleckseder, Centralbl. f. innere Med., 1905. In regard to the variation in the amount of various constituents in saliva see Fleckseder, l. c., and Tezner, Arch. internat. de Physiol., **2**.

Sulphocyanides, which, although not constant, occur in the saliva of man and certain animals, may be easily detected by acidifying the saliva with hydrochloric acid and treating with a very dilute solution of ferric chloride. As control, especially in the presence of very small quantities, it is best to compare the test with another test-tube containing an equal amount of acidulated water and ferric chloride. Other methods have been suggested by GSCHEIDLEN, SOLERA, and GANASSINI. The quantitative estimation can be done according to MUNK'S [1] method.

Ptyalin, or salivary diastase, is the amylolytic enzyme of the saliva. This enzyme is found in human saliva,[2] but not in that of all animals, especially not in the typical carnivora. It occurs not only in adults, but also in new-born infants. In opposition to ZWEIFEL'S views, BERGER [3] claims that it is present not only in the parotid gland of children, but also in the mucin glands.

According to H. GOLDSCHMIDT [4] the saliva (parotid saliva) of the horse does not contain ptyalin, but a zymogen of the same, while in other animals and man the ptyalin is formed from the zymogen during secretion. In horses the zymogen is transformed into ptyalin during mastication, and bacteria seem to give the impulse to this change. During precipitation with alcohol the zymogen is changed into ptyalin.

Ptyalin has not been isolated in a pure form up to the present time. It can be obtained purest by COHNHEIM'S [5] method, which consists in carrying the enzyme down mechanically with a calcium-phosphate precipitate and washing the precipitate with water, which dissolves the ptyalin, and from which it can be obtained by precipitating with alcohol. For the study or demonstration of the action of ptyalin one employs a watery or glycerin extract of the salivary glands, or simply the saliva itself.

Ptyalin, like other enzymes, is characterized by its action. This consists in converting starch into dextrins and sugar. Our knowledge as to the process going on here is just as uncertain as our knowledge on the formation of sugar from starch (see page 223). The nature of the sugar thus produced is known with certainty. For a long time it was considered that dextrose was the sugar formed from starch and glycogen, but SEEGEN and O. NASSE have shown that this is not true. MUSCULUS and v. MERING have shown that the sugar formed by the action of saliva,

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[1] Gscheidlen, Maly's Jahresber., **4**; Solera, see *ibid.*, **7** and **8**; Munk, Virchow's Arch., **69**; Ganassini, Biochem. Centralbl., **2**, p. 361

[2] In regard to the variation in the quantity of ptyalin in human saliva see Hofbauer, Centralbl. f. Physiol., **10**, and Chittenden and Richards, Amer. Journ. of Physiol., **1**; Schüle, Maly's Jahresber., **29**; Tezner, l. c.

[3] Zweifel, Untersuchungen über den Verdauungsapparat der Neugeborenen (Berlin, 1874); Berger, see Maly's Jahresber., **30**, 399.

[4] Zeitschr. f. physiol. Chem., **10**.

[5] Virchow's Arch., **28**.

amylopsin, and diastase upon starch and glycogen is for the most part maltose. This has been substantiated by BROWN and HERON. E. KÜLZ and J. VOGEL[1] have also demonstrated that in the saccharification of starch and glycogen, isomaltose, maltose, and some dextrose are formed, the varying quantities depending upon the amount of ferment and the length of its action. The formation of dextrose is claimed by TEBB, RÖHMANN, and HAMBURGER[2] to be only a product of the inversion of the maltose by the maltase.

The action of ptyalin in various *reactions* has been the subject of numerous investigations.[3] Natural alkaline saliva is very active, but it is not so active as when made neutral. It may be still more active under certain circumstances in faintly acid reaction, and according to CHITTENDEN and SMITH it acts better when enough hydrochloric acid is added to saturate the proteins present than when only neutralized. When the acid-combined protein exceeds a certain amount, then the diastatic action is diminished. The addition of alkali to the saliva decreases its diastatic action; on neutralizing the alkali with acid or carbon dioxide the retarding or preventive action of the alkali is arrested. According to SCHIERBECK, carbon dioxide has an accelerating action in neutral liquids, while EBSTEIN claims that it has, as a rule, a retarding action. Organic as well as inorganic acids, when added in sufficient quantity, may stop the diastatic action entirely. The degree of acidity necessary in this case is not always the same for a certain acid, but is dependent upon the quantity of ferment. The same degree of acidity in the presence of large amounts of ferment has a weaker action than in the presence of smaller quantities. Hydrochloric acid is of special physiological interest in this regard, for it prevents the formation of sugar even in very small amounts (0.03 p. m.). Hydrochloric acid has not only the property of preventing the formation of sugar, but, as shown by LANGLEY, NYLÉN, and others, may entirely destroy the enzyme. This is important in regard to the physiological significance of the saliva. According to ROGER and SIMON[4] ptyalin is not destroyed by gastric juice, but its action is

[1] Seegen, Centralbl. f. d. med. Wissensch., 1876, and Pflüger's Arch., **19**; Nasse, *ibid.*, **14**; Musculus and v. Mering, Zeitschr. f. physiol. Chem., **2**; Brown and Heron, Liebig's Annal., **199** and **204**; Külz and Vogel, Zeitschr. f. Biologie, **31**.

[2] Tebb, Journ. of Physiol., **15**; Röhmann, Ber. d. deutsch. chem. Gesellsch., **27**; Hamburger, Pflüger's Arch., **60**.

[3] See Hammarsten, Maly's Jahresber., **1**; Chittenden and Griswold, Amer. Chem. Journ., **3**; Langley, Journal of Physiol., **3**; Nylén, Maly's Jahresber., **12**, 241; Chittenden and Ely, Amer. Chem. Journ., **4**; Langley and Eves, Journal of Physiol., **4**; Chittenden and Smith, Yale College Studies, **1**, 1885, 1; Schlesinger, Virchow's Arch., **125**; Schierbeck, Skand. Arch. f. Physiol., **3**; Ebstein and C. Schulze, Virchow's Arch. **134**; Kübel, Pflüger's Arch., **56**.

[4] Compt. rend. soc. biol., **62**.

only inhibited because saliva made inactive in this manner can be reactivated by a small quantity of saliva or pancreatic juice. The experiments recorded in support of this assumption are not conclusive enough for such a view. That boiled starch (paste) is quickly, and unboiled starch only slowly, converted into sugar is also of interest. Various kinds of unboiled starch are converted with different degrees of rapidity.

Several series of investigations have been made upon the *velocity* with which ptyalin acts, and as in testing enzyme action in general, the experimenters have not made use of the different times required to produce equal chemical changes as a measure of the velocity, but have taken the quantities of substance changed in equal times. Although the results are somewhat divergent it is possible to deduce the following from them. The velocity increases, at least under conditions otherwise favorable, with the *amount of enzyme* and with an increasing *temperature* to a little above 40° C. *Foreign substances*, such as metallic salts,[1] have different effects. Certain salts, even in small quantities, completely arrest the action; for example, $HgCl_2$ accomplishes this result completely by the presence of only 0.05 p. m. Others have an accelerating action, and this seems to apply to the salts of the saliva. According to GUYENOT the saliva has a weaker action the more it is freed from salts by dialysis. On the addition of salts the dialyzed saliva becomes active again, especially on the addition of calcium or potassium chloride. ROGER[2] believes that the presence of phosphates is a necessity for the action of saliva. The amount of salts added is of special importance for the action of the saliva, and one salt, which in small quantities has an accelerting action, may in large quantities have a retarding action. As an example we can mention $MgSO_4$, but unfortunately the opinions in regard to this salt, as well as others, are widely divergent. The presence of peptone has an accelerating action on the sugar formation (CHITTENDEN and SMITH and others). The *accumulation of the products of the amylolytic decomposition* also checks the action of the saliva. This has been shown by special experiments made by SH. LEA.[3] He made parallel experiments with digestions in test-tubes and in dialyzers, and found on the removal of the products of the amylolytic decomposition by dialysis that the formation of sugar took place sooner, but also that considerably more maltose and less dextrin were formed.

To show the action of saliva or ptyalin on starch the three ordinary tests for dextrose may be used, namely, MOORE'S or TROMMER'S test or

[1] See O. Nasse, Pflüger's Arch., **11**, and Chittenden and Painter, Yale College Studies, **1**, 1885, 52; Kübel, Pflüger's Arch., **76**; Patten and Stiles, Amer. Journ. of Physiol., **17**.

[2] Guyenot, Compt. rend. soc. biol., **63**; Roger, *ibid.*, **65**.

[3] Journ. of Physiol., **11**.

the *bismuth test* (see Chapter XV). It is also necessary, as a control, to first test the starch-paste and the saliva for the presence of dextrose. The steps in the transformation of starch into amidulin, erythrodextrin, and achroödextrin may be shown by testing with iodine.

Maltase occurs in saliva to only a slight extent. It converts maltose into dextrose. According to STICKER [1] saliva also has the power of splitting sulphuretted hydrogen from the sulphur oils of radishes, onions, and certain other vegetables.

The *quantitative composition* of the mixed saliva must vary considerably, not only because of individual differences, but also because under varying conditions there may be an unequal division of the secretion from the different glands. We give herewith a few analyses of human saliva as examples of its composition. The results are in parts per 1000.

	BERZELIUS.	JACUBOWITSCH.	FRERICHS.	TIEDEMANN and GMELIN.	HERTER.	LEHMANN.	HAMMERBACHER.[2]
Water	992.9	995.16	994.1	988.3	994.7	.....	994.2
Solids	7.1	4.84	5.9	11.7	5.3	3.5–8.4 in filtered saliva.	5.8
Mucus and epithelium	1.4	1.62	2.13	.....	.....	.....	2.2
Soluble organic substances. (Ptyalin of early investigators.)	3.8	1.34	1.42	.....	3.27	.....	1.4
Sulphocyanides	.....	0.06	0.10	.....	.....	0.064 to 0.090	0.04
Salts	1.9	1.82	2.19	.....	1.30	.....	2.2

HAMMERBACHER found in 1000 parts of the ash from human saliva: potash 457.2, soda 95.9, iron oxide 50.11, magnesia 1.55, sulphuric anhydride (SO_3) 63.8, phosphoric anhydride (P_2O_5) 188.48, and chlorine 183.52.

The quantity of saliva secreted during twenty-fours hours cannot be exactly determined, but has been calculated by BIDDER and SCHMIDT to be 1400–1500 grams. The most abundant secretion occurs during meal-times. According to the calculations and determinations of TUCZEK,[3] in man 1 gram of gland yields 13 grams of secretion in the course of one hour during mastication. These figures correspond fairly well with those representing the average secretion from 1 gram of gland in animals,

[1] Münch. med. Wochenschr., **43**.

[2] Zeitschr. f. physiol. Chem., **5**. The other analyses are cited from Maly, Chemie der Verdauungssäfte, Hermann's Handbuch d. Physiol., **5**, part II, 14.

[3] Bidder and Schmidt, l. c., 13; Tuczek, Zeitschr. f. Biologie, **12**.

namely, 14.2 grams in the horse and 8 grams in oxen. The quantity of secretion per hour may be 8 to 14 times greater than the entire mass of glands, and there is probably no gland in the entire body, so far as is known at present—the kidneys not excepted—whose ability of secretion under physiological conditions equals that of the salivary glands. A remarkably abundant secretion of saliva is induced by pilocarpine, while atropine, on the contrary, inhibits it.

That the secretion of saliva, even if we do not consider such substances as ptyalin, mucin, and the like, is not a process of filtration, follows for many reasons, especially the following: The salivary glands have a specific property of eliminating certain substances, such as potassium salts (Salkowski [1]), iodine, and bromine compounds, but not others, for example, iron compounds and dextrose. It is also noticeable that the saliva is richer in solids when it is eliminated quickly by gradually increased stimulation, and in larger quantities than when the secretion is slower and less abundant (Heidenhain). The amount of salts increases also to a certain degree by an increasing rapidity of elimination (Heidenhain, Werther, Langley and Fletcher, Novi [2]).

Like the secretion processes in general, the secretion of saliva is closely connected with the processes in the cells. The chemical processes going on in these cells during secretion are still unknown.

The Physiological Importance of the Saliva. The quantity of water in the saliva renders possible the action of certain bodies on the organs of taste, and it also serves as a solvent for a part of the nutritive substances. The importance of the saliva in mastication is especially marked in herbivora, and there is no question as to its importance in facilitating the act of swallowing. The saliva containing mucin is especially important in this regard, and Pawlow's school has shown that the secretion also regulates itself in this regard. The saliva is also of importance, as it serves in washing out the mouth and thereby acts as a protection against destructive substances or bodies foreign to the mouth. The power of converting starch into sugar is not inherent in the saliva of all animals, and even when it possesses this property the intensity varies in different animals. In man, whose saliva forms sugar rapidly, a production of sugar from (boiled) starch undoubtedly takes place in the mouth, but how far this action proceeds after the morsel has entered the stomach depends upon the rapidity with which the acid gastric juice mixes with the swallowed food, and also upon the relative amounts of the gastric juice and food in the stomach. The large quantity of water which is swallowed with

[1] Virchow's Arch., **53**.

[2] Heidenhain, Pflüger's Arch., **17**; Werther, *ibid.*, **38**; Langley and Fletcher, Proc. Roy. Soc., **45**, and especially Phil. Trans. Roy. Soc. London, **180**; Novi, Arch. f. (Anat. u.) Physiol., 1888.

the saliva must be absorbed and pass into the blood, and it must in this way go through an intermediate circulation in the organism. Thus the organism possesses in the saliva an active medium by which a constant stream, conveying the dissolved and finely divided bodies, passes into the blood from the intestinal canal during digestion. The relation of the saliva or the salivary glands to the secretion of gastric juice will be mentioned in the next section.

Salivary Concrements. The so-called tartar is yellow, gray, yellowish-gray, brown or black, and has a stratified structure. It may contain more than 200 p. m. organic substances, which consist of mucin, epithelium, and LEPTOTHRIX-CHAINS. The chief part of the inorganic constituents consists of calcium carbonate and phosphate. The salivary calculi may vary in size from that of a small grain to that of a pea or still larger (a salivary calculus has been found weighing 18.6 grams), and they contain variable quantities of organic substances (50–380 p. m.), which remain on extracting the calculus with hydrochloric acid. The chief inorganic constituent is calcium carbonate.

II. THE GLANDS OF THE MUCOUS MEMBRANE OF THE STOMACH, AND THE GASTRIC JUICE.

The glands of the mucous coat of the stomach have long been divided into two distinct classes. Those which occur in the greatest abundance and which have the greatest size in the fundus are called *fundus*, rennin or pepsin glands, and the others, which occur only in the neighborhood of the pylorus, have received the name of *pyloric glands*, sometimes also, though incorrectly, called *mucous glands*. The division of these two forms of glands in the mucous membrane of the stomach is essentially different in various animals. The mucous coating of the stomach is covered throughout with a layer of columnar epithelium, which is generally considered as consisting of goblet cells that produce mucus by a metamorphosis of the protoplasm.

The **fundus glands** contain two kinds of cells: ADELOMORPHIC or chief cells, and DELOMORPHIC or COVER cells, the latter formerly called RENNIN or pepsin cells. Both kinds consist of protoplasm rich in proteins; but their relation to coloring-matters seems to show that the protein substances of both are not identical. The nucleus must consist chiefly of nuclein. Besides the above-mentioned constituents, the fundus glands contain as more specific constituents several enzymes or their zymogens, besides a little fat and cholesterin.

The **pyloric glands** contain cells which are generally considered as related to the above-mentioned chief cells of the fundus glands. As these glands were formerly thought to contain a larger quantity of mucin, they were also called mucous glands. According to HEIDENHAIN, independent of the columnar epithelium of the excretory ducts they take no part worthy of mention in the formation of mucus, which according to

his views is effected by the epithelium covering the mucous membrane. The pyloric glands also seem to contain *zymogens*. Alkali chlorides, alkali phosphates, and calcium phosphates are found in the mucous coating of the stomach.

LIEBERMANN [1] obtained an acid-reacting residue on digesting the mucosa of the stomach with pepsin-hydrochloric acid, which strangely enough contained no nuclein, but only a protein containing lecithin, called lecithalbumin. To this lecithalbumin he ascribes a great importance in the secretion of hydrochloric acid.

The Gastric Juice. The observations of HELM and BEAUMONT on persons with gastric fistula led to the suggestion that gastric fistulas be made on animals, and this operation was first performed by BASSOW [2] in 1842 on a dog. VERNEUIL performed the same on a man 1876 with successful results. PAWLOW [3] has recently improved the surgery of gastric fistula and has added much to the study of gastric secretion.

As most investigations upon gastric digestion, and also upon digestion as a whole, are based on observations upon dogs and then upon man, and for this reason, when not otherwise stated, in this chapter on the study of digestion we give the conditions in dogs and man.

The secretion of gastric juice is not continuous, at least in man and in the mammals experimented upon. It only occurs under psychic influence, and also by stimulation of the mucous membrane of the stomach or the intestine. The most exhaustive researches on the secretion of gastric juice (in dogs) have been made by PAWLOW and his pupils.

In order to obtain gastric juice free from saliva and food residues they arranged besides a gastric fistula also an œsophageal fistula from which the swallowed food could be withdrawn with the saliva without entering the stomach, and in this manner an apparent or sham feeding was possible. In this way it was possible to study the influence of psychical moments on one side and the direct action of food on the mucous membrane on the other. After a method suggested by HEIDENHAIN and later improved by PAWLOW and CHIGIN, they have succeeded in preparing a blind sac by partial dissection of the fundus part of the stomach, and the secretion processes could be studied in this sac while the digestion in the other parts of the stomach was going on. In this way they were able to study the action of different foods on the secretion.

The most essential results of the investigations of PAWLOW and his pupils are as follows: Mechanical stimulation of the mucosa does not produce any secretion. Mechanical irritation of the mucous membrane

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[1] Pflüger's Arch., **50**.

[2] Helm, Zwei Krankengeschichten, Wien, 1803, cited from Hermann's Handbuch, **5**, part II, 39; Beaumont, "The Physiology of Digestion," 1833; Bassow, Bull. de la soc. des natur. de Moscou, **16**, cited from Maly in Hermann's Handbuch, **5**, 38; Verneuil, see Ch. Richet, "Du suc gastrique chez l'homme," etc. (Paris, 1878), 158.

[3] Pawlow, Die Arbeit der Verdauungsdrüsen (Wiesbaden, 1898), where the works of his pupils are also mentioned. See also Ergebnisse der Physiologie, **1**, Abt. 1.

of the mouth causes no reflex excitation of the secretory nerves of the stomach. There are two moments which cause a secretion, namely, the psychical moment—the passionate desire for food and the sensation of satisfaction and pleasure on partaking it—and the chemical moment, the action of certain chemical substances on the mucous membrane of the stomach. The first moment is the most important. The secretion occurring under its influence by the vagus fibers appears earlier than that produced by chemical irritants, but only after an interval of at least 4½ minutes. This secretion is more abundant but less continuous than the "chemical." It yields a more acid and active juice than the latter. As chemical excitants which cause a secretion reflexively through the stomach mucosa we include water (slight action) and certain unknown extractive substances contained in meat and meat extracts, in impure peptone, and also, it seems, in milk. According to HERZEN and RADZIKOWSKI [1] and others, alcohol is also a strong agent in producing a flow of juice. The claims in regard to the action of sodium chloride and alkali carbonates are somewhat disputed. That the alkali carbonates retard or inhibit secretion is the opinion of many, but from recent determinations [2] it would seem as if the concentration of the carbonate as well as of sodium chloride exercises a certain influence, so that a weaker concentration is indifferent or retarding, while somewhat stronger concentration has an accelerating action upon secretion, though investigators are not agreed as to results. Bitter substances partaken of in small amounts a certain time before a meal increase the secretion, while larger amounts have a retarding action (BORISSOW, STRASHESKO [3]). Fats have a retarding action on the appearance of secretion and diminish the quantity of juice secreted as well as the amount of enzyme. The substances, such as egg-albumin, which act as chemical stimulants, cannot be digested by the "psychical" secretion, but may perhaps cause a chemical secretion by their decomposition products.

The quantity of juice secreted during digestion is proportional to the quantity of food, and the secretion of gastric juice may also be influenced by the kind of food. This action of various foods—meat, bread, and milk—may be arranged in progressive series as follows:

	Acidity.	Digestive Activity.	Duration of Secretion.
1.	Meat.	Bread.	Bread.
2.	Milk.	Meat.	Meat.
3.	Bread.	Milk.	Milk.

[1] Pflüger's Arch., **84**, 513.

[2] See Rozenblatt, Bioch. Zeitschr., **4**; Mayeda, *ibid.*, **2**; Pimenow, Bioch. Centralbl., **6**; Lönquist, Maly Jahresb., **36**.

[3] Borissow, Arch. f. exp. Path. u. Pharm., **51**; Strashesko, see Biochem. Centralbl., **4**, 148.

The acidity is greatest with a meat diet and lowest with bread; the quantity of enzyme is, on the contrary, highest with a bread diet and lowest with milk.

The secretion in the stomach may also be influenced by the small intestine, and in this way, as shown by the investigations of PAWLOW and his pupils, the fats have a retarding action upon the secretion of juice and upon digestion by acting reflexly upon the duodenal mucosa. In dogs on feeding fat (oil) with food containing starch, the secretion of gastric juice remains reduced during the entire period of feeding, and fat in connection with protein food has a similar action, with the exception that in this case the retarding action is observed only in the first hours of digestion. According to PIONTKOWSKI [1] the oil-soaps differ from the neutral fats by having a strong action on the flow of juice, and this is the reason why about five to six hours after a meal with fat the secretion of juice is stopped, as just at this time the soaps are being formed. According to FROUIN the food in the intestine produces a secretion of gastric juice which continues after the action of the psychic moment has ceased. LECONTE [2] arrived at similar results, and he ascribes a less subordinate importance to the chemical secretion as compared with the psychic secretion, than PAWLOW does.

The behavior of the different parts of the stomach in secretion is also of interest. The work of PAWLOW and his pupils GROSS and KRYSHYSCHKOWSKY [3] have shown that meat and its extractives as well as the digestion products and milk especially act upon the pyloric part, although not entirely, while they are inactive upon the fundus. Alcohol also acts upon the fundus part. In close relation to what has been said above stands the observation of EDKINS that the pylorus part of the stomach contains a substance, a *prosecretin*, which by acids and certain other substances is transformed into a *secretin*, which when introduced into the blood circulation causes a secretion of gastric juice. HEMMETER,[4] claims that a secretin for the secretion of gastric juice is also produced in the salivary glands. The extirpation of all the salivary glands in dogs causes a marked diminution in the secretion of gastric juice, while the intravenous or peritoneal injection of an extract of the salivary glands of dogs produces a secretion of gastric juice.

We know very little positively in regard to the gastric secretion in man. According to the earlier authorities the irritants may be mechanical, thermic, and chemical. Among the chemical excitants we include alcohol and ether, which in too great a concentration bring about no

[1] See Biochem. Centralbl., **3**, 660.

[2] Frouin, Compt. rend. soc. biol., **53**; Leconte, La Cellule, **17**.

[3] Gross, Bioch. Centralbl., **5**, 669; Kryshyschkowsky, Maly's Jahresb., **36**, 403.

[4] Edkins, Journ. of Physiol., **34**; Hemmeter, Bioch. Zeitschr., **11**.

physiological secretion, but rather the transudation of a neutral or faintly alkaline fluid. Certain acids, such as carbonic acid, neutral salts, meat extracts, spices, and other bodies also belong to this group. The reports on this subject are unfortunately very uncertain and contradictory.

The question as to how far the observations made by PAWLOW and his school can be applied to man is of special interest. Many observations on this question have been collected [1] and they compare favorably with the observations made upon dogs. Thus in man a psychic secretion of gastric juice can also be brought about, and it has also been observed that it can be stopped by emotions. As in dogs, so also in man, after sham feeding, a secretion takes place after a pause, whose duration varies in different cases. In some cases, as in dogs after meat feeding, the pause was about five minutes. The chewing of indifferent bodies did not affect the glands, while bodies acting upon the organs of smell and taste had an exciting action. UMBER observed besides this, that after the introduction of a nutritive enema into the rectum a secretion of gastric juice was produced by reflex action.

From these observations of HORNBORG and UMBER, as well as from some earlier observations of SHÜLE, TROLLER, RIEGEL, and SCHEUER,[2] we conclude that in man the psychic secretion is much less than that produced by the introduction of food or bodies having a pleasant taste. That the preparation of the food in the mouth has an essential influence upon the secretion is proven without doubt, but we are not agreed as to how this action takes place. Certain experimenters consider the secreted and swallowed saliva as the most essential factor in this action, while others believe that the act of chewing, and still others that the chemical action and the sense of taste, are the most important.

In regard to the action of saliva HEMMETER finds that after the extirpation of the salivary glands, the introduction into the stomach of chewed food soaked with dog-saliva, has no special action upon the secretion of juice. On the other hand FROUIN [3] observed that the introduction of saliva into the large stomach of dogs acts favorably upon the secretion in the small stomach (see p. 437), and the acidity as well as the digestive activity of the juice is increased. This action does not depend, according to FROUIN, upon the alkali of the saliva.

The Qualitative and Quantitative Composition of the Gastric Juice. The human gastric juice, which can seldom be obtained pure and free

[1] Hornborg, Maly's Jahresb., **33**, 547; Umber, Berl. klin. Wochenschr., 1905; Cade and Latarjet, Compt. rend. soc. biol., **57**; Kaznelson, Pflüger's Arch., **118**; Bogen, *ibid.*, **117**; Bickel, Deutsch. med. Wochenschr., **32**, and Maly's Jahresb., **36**, 411.

[2] The literature may be found in Umber's work, l. c.

[3] Compt. rend. soc. biol., **62**.

from residues of the food or from mucus and saliva, is a clear, or only very faintly cloudy, and nearly colorless fluid of an insipid, acid taste and strong acid reaction. It contains, as form-elements, *glandular cells* or their *nuclei, mucus-corpuscles,* and more or less changed *columnar epithelium.*

The acid reaction of the gastric juice depends on the presence of free acid, which, as has been learned from the investigations of C. SCHMIDT, RICHET, and others, consists, when the gastric juice is pure and free from particles of food, chiefly or in large part of *hydrochloric acid.* CONTEJEAN [1] regularly found traces of lactic acid in the pure gastric juice of fasting dogs. After partaking of food, especially after a meal rich in carbohydrates, lactic acid occurs abundantly, and sometimes acetic and butyric acids. In new-born dogs the acid of the stomach is lactic acid, according to GMELIN.[2] The quantity of free hydrochloric acid in the gastric juice is, according to PAWLOW and his pupils, in dogs 5–6 p. m., and in cats an average of 5.20 p. m. HCl. In man the results obtained are variable but regularly much lower. Since it has been possible to obtain pure human gastric juice for investigation it has been found (UMBER, HORNBORG, BICKEL, SOMMERFELD [3]) that the amount of hydrochloric acid is about 4–5 p. m. There is hardly any doubt that at least a part of the hydrochloric acid of the gastric juice does not exist free in the ordinary sense, but combined with organic substances. The results obtained in testing for the acidity of gastric juice by physical methods are nearly identical with those obtained by titration (P. FRÄNCKEL [4]).

As chief organic constituent, perfectly fresh gastric juice (of dogs) contains a very complex substance (or perhaps a mixture of substances) which coagulates on boiling and which separates on strongly cooling the juice. This substance is considered, by certain experimenters (NENCKI and SIEBER, and PAWLOW) as the conveyor of the several ferment actions of the gastric juice, i.e., the pepsin as well as the rennin action. It contains lecithin and chlorine, and yields nucleoprotein, proteose, purine bases, and pentose as cleavage products (NENCKI and SIEBER [5]).

The specific gravity of gastric juice is low, 1.001–1.010. It is correspondingly poor in solids. Earlier analyses of gastric juice from man, the dog, and the sheep were made by C. SCHMIDT.[6] As these analyses

[1] Bidder and Schmidt, Die Verdauungssäfte, etc., **44**; Richet, l. c.; Contejean, Contributions a l'étude de la physiol. de l'estomac, Thèses, Paris, 1892.

[2] Pflüger's Arch., **90** and **103**.

[3] See Richet, l. c.; Contejean, l. c.; Verhaegen, "La Cellule," 1896 and 1897; Sommerfeld, Bioch. Zeitschr. **9**, and also footnote 1, page 440, and the literature on the estimation of hydrochloric acid in the gastric juice contents (p. 465).

[4] Zeitschr. f. exp. Path. u. Therap., **1**.

[5] Zeitschr. f. physiol. Chem., **32**.

[6] l. c.

refer only to impure gastric juice they are of little value. Rosemann,[1] who has investigated the gastric juice secreted by a dog after sham feeding found an average of 4.22 p. m. solids, among which 1.32 p. m. were mineral bodies and about 2.90 p. m. organic substance. The amount of nitrogen in one case was 0.36 p. m., in another 0.54 p. m. and the quantity of HCl was about 5.6 p. m. The ash consisted chiefly of potassium chloride, namely 980–990 p. m. of the inorganic part. Nencki and Sieber[2] found 3.06 p. m. solids in the pure gastric juice of a dog. Nencki[3] found 5 milligrams sulphocyanic acid in a liter of gastric juice of a dog.

In the ash of human gastric juice after sham-feeding Albu[4] found 356.2 p. m. K_2O; 226.5 p. m. Na_2O, and 497.3 p. m. Cl. The amount of salts insoluble in water was 23.9 p. m. In hyperacidity he found nearly the same composition.

Besides the free hydrochloric acid, *pepsin*, *rennin*, and a *lipase* are the other physiologically important constituents of gastric juice.

Pepsin. This enzyme is found, with the exception of certain fishes, in all vertebrates thus far investigated.

Pepsin occurs in adults and in new-born infants. This condition is different in new-born animals. While in a few herbivora, such as the rabbit, pepsin occurs in the mucous coat before birth, this enzyme is entirely absent at the birth of those carnivora which have thus far been examined, such as the dog and cat.

In various invertebrates enzymes have also been found which have a proteolytic action in acid solutions. It has been shown that these enzymes, nevertheless, are not in all animals identical with ordinary pepsin. According to Klug and Wróblewski[5] the pepsins found in man and various higher animals are somewhat different, an observation which according to the experience of Hammarsten is very probable. Enzymes also occur in various plants and animal organs, although not identical with pepsin, but which act in acid reaction. The enzyme obtained from the Nepenthes, which dissolves proteins only in acid reaction, stands very close to pepsin. An enzyme more closely related to trypsin or erepsin (see sections III and IV) is, on the contrary, Glaessner's *pseudopepsin*, which according to him is the only peptic enzyme in the pyloric end. Pseudopepsin, whose existence is disputed by Klug, while others (Reach, Pekelharing) affirm its occurrence in the mucous membrane, cannot, according to Hammarsten, either be the only or the most prominent peptic enzyme of the pyloric part. According to Glaessner, it also

[1] Pflüger's Arch., **118**.
[2] Zeitschr. f. physiol. Chem., **32**.
[3] Ber. d. d. Chem. Gesellsch., **28**.
[4] Zeitschr. f. Path. u. Therap., **5**.
[5] Klug, Pflüger's Arch, **60**; Wróblewski, Zeitschr. f. physiol. Chem., **21**.

acts in neutral and alkaline reaction and yields tryptophane among other cleavage products. According to BERGMANN [1] it is identical with erepsin (see below). Among the enzymes of the mucosa of the stomach belongs the so-called *antipepsin* discovered by WEINLAND,[2] which has a retarding action upon pepsin digestion and, as some claim, prevents the self-digestion of the mucous membrane.

Pepsin is as difficult to isolate in a pure condition as are other enzymes. The pepsin prepared by BRÜCKE and SUNDBERG gave negative results with most reagents for proteins, and showed nevertheless a powerful action, which seems to indicate that it was very pure. SCHOUMOW-SIMANOWSKI, NENCKI and SIEBER, and also PEKELHARING, have designated as the true enzyme the substance containing chlorine, which they obtained by strongly cooling the gastric juice. That this precipitate is not a chemical individual, and hence cannot be pepsin, follows from the investigations of PEKELHARING. While pepsin, according to NENCKI and SIEBER, was rich in phosphorus and contained nucleoprotein, PEKELHARING'S pepsin was free from phosphorus and yielded no nucleoprotein. FRIEDENTHAL and MIYAMOTA [3] have also shown that the pepsin is still active after the splitting off of the nuclein complex (and also the protein). As pepsin is readily precipitated with the proteins and combines therewith, it is difficult to decide whether pepsin is a protein substance or not, and the question as to its nature is still undecided, just as is the case with other enzymes. As ordinarily known, pepsin, at least in an impure form, is soluble in water and glycerin. It is precipitated by alcohol, but is only slowly destroyed thereby. In aqueous solution its action is quickly destroyed on heating to boiling. According to BIERNACKI [4] pepsin in neutral solutions is destroyed by heating to 55° C. In the dry state it can be heated to over 100° C. without losing its activity. In the presence of 2 p. m. HCl a temperature of 55° C. is not injurious, and the compound with acid is more resistant than the free pepsin (GROBER [5]). Pepsin in acid solution is destroyed by heating to 65° C. for five minutes. On adding peptone or certain salts the pepsin may be heated to 70° C. for the same time without destruction.

The behavior of pepsin on heating its acid solution is influenced not

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[1] Glaessner, Hofmeister's Beiträge, **1**; Klug, Pflüger's Arch., **92**; Reach, Hofmeister's Beiträge, **4**; Pekelharing, Arch. des scienc. biolog., St. Pétersbourg, **11**; Pawlow-Festband, 1904; Bergmann, Skand. Arch. f. Physiol., **18**.

[2] Zeitschr. f. Biologie, **44**.

[3] Brücke, Wien. Sitzungsber., **43**; Sundberg, Zeitschr. f. physiol. Chem., **9**; Schoumow-Simanowski, Arch. f. exp. Path. u. Pharm., **33**; Pekelharing, Zeitschr. f. physiol. Chem., **22** and **35**; Nencki and Sieber, *ibid.*, **32**; Friedenthal and Miyamota, Centrabl. f. Physiol., **15**, 785.

[4] Zeitschr. f. Biologie, **28**.

[5] Arch. f. exp. Path. u. Pharm., **51**.

only by the degree of acidity, but by the duration of heating and also by the amount of other bodies in the solution. If an acid (0.2 per cent HCl) infusion of the calf's stomach be warmed for several days to about 40° or 45° C., a part of the pepsin is destroyed, but we obtain in this manner an infusion which still dissolves proteins but has no rennin action (HAMMARSTEN [1]). The pepsin from different animals acts differently in this regard and the pepsin of the pike stomach is very quickly destroyed at 37–40° C.

Pepsin is extraordinarily sensitive to the action of alkalies, not only caustic, and carbonated, but also against the hydroxides of the alkaline earths. It is easily made inactive by these substances. If the action of the alkali is not too strong then, as shown by PAWLOW and TICHOMIROW,[2] the enzyme can in part be reactivated by the addition of acid if the greater part (about four-fifths), of the alkalinity be neutralized by the addition of acid and then after some hours more acid be added. If the entire quantity of acid be added at one time the reaction does not take place.

The only property which is characteristic of pepsin is that it dissolves protein bodies in acid but not in neutral or alkaline solutions, with the formation of proteoses, peptones, and other products.

The methods for the preparation of relatively pure pepsin depend, as a rule, upon its property of being thrown down with finely divided precipitates of other bodies, such as calcium phosphate or cholesterin. The rather complicated methods of BRÜCKE and SUNDBERG are based upon this property. PEKELHARING makes use of a prolonged dialysis and precipitation with 0.2 p. m. HCl.

Very permanent pepsin solutions, from which the enzyme with considerable protein can be precipitated by alcohol, may be prepared by extraction with glycerin. Solutions having a strong action may also be prepared by making an infusion of the gastric mucosa of an animal in acidified water (2–5 p. m. HCl). This is unnecessary, as we can obtain pure gastric juice according to PAWLOW'S method, and also because very active commercial preparations of pepsin can be bought in the market.

The Action of Pepsin on Proteins. Pepsin is inactive in neutral or alkaline reactions, but in acid liquids it dissolves coagulated protein bodies. The protein always swells and becomes transparent before it dissolves. Unboiled fibrin swells up in a solution containing 1 p. m. HCl, forming a gelatinous mass, and does not dissolve at ordinary temperature within a couple of days. Upon the addition of a little pepsin, however, this swollen mass dissolves quickly at ordinary temperatures. Hard-boiled egg albumin, cut in thin pieces with sharp edges, is not perceptibly changed by dilute acid (2–4 p. m. HCl) at the temperature of the body in the course of several hours. But the simultaneous presence

[1] Zeitschr. f. physiol. Chem., 56. [2] *Ibid.*, 55.

of pepsin causes the edges to become clear and transparent, blunt and swollen, and the protein gradually dissolves.

From what has been said above in regard to pepsin, it follows that proteins may be employed as a means of detecting pepsin in liquids. Ox-fibrin may be employed as well as coagulated egg-albumin, which latter is used in the form of slices with sharp edges. As the fibrin is easily digested at the normal temperature, while the pepsin test with egg-albumin requires the temperature of the body, and as the test with fibrin is somewhat more delicate, it is often preferred to that with egg-albumin. When we speak of the "*pepsin test*" without further explanation we ordinarily understand it as the test with fibrin.

This test, nevertheless, requires care. The fibrin used should be ox-fibrin and not pig-fibrin, which last is dissolved too readily with dilute acid alone. The unboiled ox-fibrin may be dissolved by acid alone without pepsin, but this generally requires more time. In testing with unboiled fibrin at normal temperature, it is advisable to make a control test with another portion of the same fibrin with acid alone. Since at the temperature of the body unboiled fibrin is more easily dissolved by acid alone, it is best always to work with boiled fibrin.

As pepsin has not thus far been prepared in a positively pure condition, it is impossible to determine the absolute quantity of pepsin in a liquid. It is possible only to compare the relative amounts of pepsin in two or more liquids, which may be done in several ways.

The older method, that of BRÜCKE, consists in diluting the two pepsin solutions to be compared with certain proportions of 1 p. m. hydrochloric acid, so that when the amount of pepsin contained in the original solution is equal to 1, each solution contains a degree of dilution, p, corresponding to 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, etc. A flock of fibrin or a piece of hard-boiled egg is added to each test and the time noted when each test begins to digest and when it ends. The relative amount of pepsin is calculated from the rapidity of digestion as follows: The tests $p=\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, of one series is digested in the same time as tests $p=1$, $\frac{1}{2}$, $\frac{1}{4}$ of the other series, hence the first solution contained four times as much pepsin. GRÜTZNER [1] has improved this test by using fibrin colored with carmine, and on comparing with carmine solutions of known dilution he determines colorimetrically the rapidity of digestion.

METT'S Method. Draw up white of egg in a glass tube 1–2 millimeters in diameter, coagulate it by plunging it into water at 95° C., and cut the ends off sharply; then add two tubes to each test-tube with a few cubic centimeters of the acid pepsin solution; allow them to digest at body temperature, and after a certain time, generally after ten hours, measure the lineal extent of the digested layer of albumin in the various tests, bearing in mind that the digested layer at each end must not be longer than 6–7 millimeters. The quantity of pepsin in the comparative tests is as the square of the millimeters of the albumin-column dissolved in the same time. Thus if in one case 2 millimeters of albumin were dissolved and in the other 3 millimeters, then the quantity of pepsin is as 4:9. If the fluid removed from the stomach, which is rich in bodies having a disturb-

[1] Grützner, Pflüger's Arch., **8** and **106**. See also A. Korn, "Ueber Methoden Pepsin quantitativ zu bestimmen," Inaug.-Dissert., Tübingen, 1902.

ing influence upon pepsin digestion, is to be tested, then the liquid must be first properly diluted with 2–4 p. m. hydrochloric acid (NIERENSTEIN and SCHIFF [1]).

Objections have been raised against these methods from several sides, especially by GRÜTZNER, but they can be recommended for practical purposes as being simple and rather accurate. HUPPERT and E. SCHÜTZ measure the relative quantities of pepsin from the amount of secondary proteoses formed under certain conditions. The proteoses were determined by the polariscope. J. SCHÜTZ determines the total proteose-nitrogen, and SPRIGGS [2] finds that the change in the viscosity is a measure of the amount of pepsin.

VOLHARD and LÖHLEIN [3] use an acid casein solution for the pepsin determination, and determine, after precipitation with sodium sulphate, the acidity of the filtrate of the digested test as well as of the original control solution. The casein is precipitated as an acid compound by the sulphate, and the filtrate separated from the precipitate contains less acid than the original solution. In proportion as the digestion progresses less substance is precipitated by the sulphate, and the acidity of the filtrate becomes correspondingly higher. The increase in acidity in the different portions varies within certain limits as the square root of the quantity of ferment.

JACOBY suggested a method which is based on the fact that a cloudy solution of ricin becomes clear by the action of pepsin-hydrochloric acid, and indeed with varying rapidity with different quantities of pepsin. This method, which requires further testing, seems to be delicate and is of value, as is doubtless the following method of FULD and LEVISON.[4] This is based on the property that edestan can be precipitated from acid solution by NaCl, but not the proteoses formed therefrom:

A solution of 1 p. m. edestin in hydrochloric acid ($\frac{3}{10}$ normal) is prepared whereby the edestin is changed into edestan. The activity of a gastric juice (or a pepsin-hydrochloric acid solution) is tested in the following manner: the solution to be tested is placed in decreasing quantities in a series of test-tubes and allowed to act upon an equal quantity of the edestan solution, 2 cc., and the minimum of juice determined which is necessary to digest the solution, within one-half an hour and at room temperature, so that on the addition of solid NaCl and shaking no precipitate occurs. GROSS [5] suggested a similar method by using an acid casein solution and precipitating with sodium acetate.

The *rapidity of the pepsin digestion* depends on several circumstances. Thus *different acids* are unequal in their action; hydrochloric acid shows in slight concentration, 0.8–1.8 p. m., a more powerful action than any other acid, whether inorganic or organic. In greater concentration other acids may have a powerful action; but no constant relation has been found between the strength of various acids and their action in pepsin digestion, and the reports of the action of different acids are contradictory.[6] Sulphuric acid, it seems, has a weaker action than the other

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[1] Mett, see Pawlow, l. c., 31; Nierenstein and Schiff, Berl. klin. Wochenschr., **40**; Jastrowitz, Bioch. Zeitschr., **2**.

[2] Huppert and Schütz, Pflüger's Arch., **80**; J. Schütz, Zeitschr. f. physiol. Chem., **30**; Spriggs, *ibid.*, **35**.

[3] Hofmeister's Beiträge, **7**.

[4] Jacoby, Bioch. Zeitschr., **1**; Fuld and Levison, *ibid.*, **6**.

[5] Berl. klin. Wochenschr., **45**.

[6] See Wróblewski, Zeitschr. f. physiol. Chem., **21**, and especially Pfleiderer, Pflüger's Arch., **66**, which also gives references to other works; Larin, Biochem. Centrabl., **1**, 484; and A. Pick, Wien. Sitzungsber., M. N. Klasse, **112**.

inorganic acids. The *degree of acidity* is also of the greatest importance. With hydrochloric acid the degree of acidity is not the same for different protein bodies. For fibrin it is 0.8–1 p. m., for myosin, casein, and vegetable proteins about 1 p. m., for coagulated egg-albumin, on the contrary, about 2.5 p. m. The rapidity of the digestion increases, at least to a certain point, with the *quantity of pepsin* present, unless the pepsin added is contaminated by a large quantity of the products of digestion, which may prevent its action.

According to E. SCHÜTZ,[1] whose observations have been confirmed by several others, the digestion products obtained in a certain time are, within certain limits, proportional to the square root of the relative amounts of pepsin (the SCHÜTZ-BORISSOW rule) while, as explained in detail in Chapter II, under certain conditions another rule exists where the quantity digested increases in proportion to the quantity of enzyme. The *kind of protein* is of importance, for example, for besides what was said above in regard to the fibrin, hard-boiled egg albumin is much easier digested by an acidity of 1-2 p. m. HCl than liquid egg albumin, which is rather resistant to the action of gastric juice.

The *accumulation of products of digestion* has a retarding action on digestion, although, according to CHITTENDEN and AMERMAN [2] the removal of the digestion products by means of dialysis does not essentially change the relation between the proteoses and true peptones. Pepsin acts more slowly at low temperatures than it does at higher ones. It is even active in the neighborhood of 0° C., but digestion takes place very slowly at this temperature. With increasing temperature the rapidity of digestion also increases until about 40° C., when the maximum is reached. According to the investigations of FLAUM [3] it is probable that the relation between proteoses and peptones remains the same, irrespective of whether the digestion takes place at a low or high temperature, so long as the digestion is continued for a long enough time. If the *swelling up of the protein* is prevented, as by the addition of neutral salts, such as NaCl, in sufficient amounts, or by the addition of bile to the acid liquid, digestion can be prevented to a greater or less extent. *Foreign bodies* of different kinds produce dissimilar effects, in which naturally the variable quantities in which they are added are of the greatest importance. Salicylic acid and carbolic acid, and especially sulphates (PFLEIDERER), retard digestion, while arsenous acid promotes it (CHITTENDEN), and hydrocyanic acid is relatively indifferent. By experiments with salt solutions so strongly diluted that the action, on account of the strong dissociation, was brought about by ions and not by the electrolytically neutral molecules (min. $\frac{1}{40}$ and max. $\frac{1}{4}$ normal salt solutions), J. SCHÜTZ [4]

[1] Zeitschr. f. physiol. Chem., 9.
[2] Journ. of Physiol., 14.
[3] Zeitschr. f. Biologie, 28.
[4] Hofmeister's Beiträge, 5.

found that the anions had a much greater retarding action upon pepsin digestion than the cations. Of these latter the sodium cation had the strongest retarding action. Alcohol in large quantities (10 per cent and above) disturbs the digestion, while small quantities act indifferently. Metallic salts in very small quantities may indeed sometimes accelerate digestion, but otherwise they tend to retard it. The action of metallic salts in different cases can be explained in various ways, but they often seem to form with proteins insoluble or difficultly soluble combinations. The alkaloids may also retard the pepsin digestion (CHITTENDEN and ALLEN [1]). A very large number of observations have been made in regard to the action of foreign substances on artificial pepsin digestion, but as these observations have not given any direct result in regard to the action of these same substances on natural digestion, as well as upon secretion and absorption, we will not discuss them here.

The Products of the Digestion of Proteins by Means of Pepsin and Acid. In the digestion of nucleoproteins or nucleoalbumins an insoluble residue of nuclein or pseudonuclein always remains, although under certain circumstances a complete solution may occur. Fibrin also yields an insoluble residue, which consists, at least in great part, of nuclein, derived from the form-elements inclosed in the blood-clot. This residue which remains after the digestion of certain proteins was called *dyspeptone* by MEISSNER. This name is therefore not only unnecessary but indeed erroneous, as this residue does not consist of bodies related to the peptones. In the digestion of proteins, substances similar to acid albuminates, *parapeptone* (MEISSNER [2]), *antialbumate*, and *antialbumid* (KÜHNE), may also be formed. On separating these bodies the filtered liquid, neutralized at boiling-point, contains *proteoses* and *peptones* in the old sense, while the so-called KÜHNE true peptone and the other cleavage products are obtained only after a longer and more intense digestion. The relation, between the proteoses, changes very much in different cases and in the digestion of the proteins. For instance, a larger quantity of primary proteoses is obtained from fibrin than from hard-boiled egg-albumin or from the proteins of meat; and the different proteins, according to the researches of KLUG,[3] yield on pepsin digestion unequal quantities of the various digestive products. In the digestion of unboiled fibrin an intermediate product may be obtained in the earlier stages of the digestion—a globulin which coagulates at

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[1] Studies from the Lab. Physiol. Chem. Yale University, **1**, 76. See also Chittenden and Stewart, *ibid.*, **3**, 60.

[2] The works of Meissner on pepsin digestion are found in Zeitschr. f. rat. Med., **7**, **8**, **10**, **12**, and **14**.

[3] Pflüger's Arch., **65**.

55° C. (HASEBROEK [1]). For information in regard to the different proteoses and peptones which are formed in pepsin digestion see pages 127 to 136.

Action of Pepsin-Hydrochloric Acid on Other Bodies. The *gelatin-forming substances* of the connective tissue, of the cartilage, and of the bones, from which last the acid dissolves only the inorganic substances, is converted into *gelatin* by digesting with gastric juice. The gelatin is further changed so that it loses its property of gelatinizing and is converted into gelatoses and peptone (see page 119). True *mucin* (from the submaxillary) is dissolved by the gastric juice, yielding substances similar to peptone, and a reducing substance similar to that obtained by boiling with a mineral acid. *Mucoids* from tendons, cartilage, and bones dissolve, according to POSNER and GIES,[2] in pepsin-hydrochloric acid, but leave a residue which amounts to about 10 per cent of the original material and which, as it seems, consists in great part, if not entirely, of a combination of proteid with glucothionic acid (Chapters VII and VIII). The solution contains primary and secondary mucoproteoses and mucopeptones. The former contain glucothionic acid, but the latter do not. *Elastin* is dissolved more slowly and yields the above-mentioned substances (page 116). *Keratin* and the epidermal formations are insoluble. The *nucleins* are dissolved with difficulty, and the cell nuclei, therefore, remain in great part undissolved in the gastric juice. The *animal cell-membrane* is, as a rule, more easily dissolved the nearer it stands to elastin, and it dissolves with greater difficulty the more closely it is related to keratin. The *membrane of the plant-cell* is not dissolved. *Oxyhæmoglobin* is changed into hæmatin and protein, the latter undergoing further digestion. It is for this reason that blood is changed into a dark-brown mass in the stomach. The gastric juice does not act upon *fat*, but, on the contrary, dissolves the cell-membrane of fatty tissue, setting the fat free. Gastric juice has no action on starch or the simple varieties of sugar. The statements in regard to the ability of gastric juice to invert cane-sugar are very contradictory. At least this action of the gastric juice is not constant, and if it is present at all it is probably due to the action of the acid.

Pepsin alone, as above stated, has no action on proteins, and an acid of the intensity of the gastric juice can only very slowly, if at all, dissolve coagulated albumin at the temperature of the body. Pepsin and acid together not only act more quickly, but qualitatively they act otherwise than the acid alone, at least upon dissolved protein. This has led to the assumption of the presence of a *pepsin-hydrochloric acid* whose existence and action are only hypothetical. As pepsin digestion, it seems, yields finally the same products as the hydrolytic cleavage with acids, we can say for the present only that this enzyme acts like other catalysts in very powerfully accelerating a process which would also proceed without the catalytes.

[1] Zeitschr. f. physiol. Chem., **11**.

[2] Amer. Journ. of Physiol., **11**.

Chymosin (RENNIN) and **Parachymosin.** Besides the enzyme called rennin (chymosin) which is found in the calf's stomach, there exists, according to BANG,[1] another chymosin, the parachymosin, which is the rennet enzyme of the pig and human stomach. This latter occurs in the gastric juice of man under physiological conditions, but may be absent under special pathological conditions (SCHUMBURG, BOAS, JOHNSON, KLEMPERER[2]). Chymosin is habitually found in the neutral, watery infusion of the fourth stomach of the calf and sheep, especially in an infusion of the fundus part. In other mammals and in birds it is seldom found, and in fishes hardly ever in the neutral infusion. In these cases, as in man and the higher animals, a rennin-forming substance, a *rennin zymogen*, occurs, which is converted into rennin by the action of an acid (HAMMARSTEN). We have no knowledge as to whether the rennet enzyme found in different animals is chymosin or parachymosin. That the chymosin occurring in the calf's stomach is not identical with that found in certain animals, for example the pike, follows without question from the experience of HAMMARSTEN. Enzymes acting like rennin are also found in the blood and several organs of higher animals, as well as in invertebrates. Similar enzymes also occur widely diffused in the plant kingdom, and numerous micro-organisms have the power of producing rennin enzymes. Antibodies to the rennet enzymes, *anti-rennins*, also occur in the animal kingdom, as shown by HAMMARSTEN and RÖDÉN in blood-serum, and may be produced in the animal body by the introduction of rennin into the latter (MORGENROTH[3]).

Rennin is just as difficult to prepare in a pure state as the other enzymes. The purest rennin enzyme thus far obtained did not give the ordinary protein reactions. On heating its solution rennin is more or less quickly destroyed, depending upon the length of heating and upon the concentration. If an active and strong infusion of the gastric mucosa in water containing 3 p. m. HCl is heated to 40–45° C. for 48 hours, the rennin is destroyed, while the pepsin remains. A pepsin solution free from rennin can be obtained in this way. Rennin is characterized by its physiological action, which consists in coagulating milk or a casein solution containing lime, if neutral or very faintly alkaline. The law of the action of this enzyme is different from that of the action of pepsin. As

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[1] Deutsch. med. Wochenschr., 1899, and Pflüger's Arch., **79**.

[2] Schumburg, Virchow's Arch., **97**. A good review of the literature may be found in Szydlowski, Beiträge zur Kenntnis des Labenzym nach Beobachtungen an Säuglingen, Jahrb. f. Kinderheilkunde (N. F.), **34**. See also Lörcher, Pflüger's Arch., **69**, which also contains the pertinent literature. An excellent review of the literature on rennin and its action may be found in E. Fuld, Ergebnisse der Physiol., **1**, Abt. 1, 468.

[3] See Rödén, Upsala Läkaref. Förh., **22**; Morgenroth, Centrabl. f. Bakter., **26** and **27**.

specially shown by FULD,[1] within certain limits, the coagulation t, multiplied by the quantity of rennin, l, equals a constant, k. As shown by BANG, this law does not apply to parachymosin.

Parachymosin differs from chymosin by being much more resistant toward acids, but is more readily destroyed by alkalies. Calcium chloride accelerates the casein coagulation with parachymosin very much more than with chymosin. GEWIN[2] has raised objections to these points of difference between chymosin and parachymosin, although the proofs that he puts forth are still not so conclusive that we have sufficient ground to doubt the existence of parachymosin.

A much-discussed question is, whether the digestion of protein and the rennet action are brought about by two special enzymes, or represent two different enzyme actions, or whether there is only one enzyme, the pepsin, which has both actions. The supporters of this last view dispose of the question in different ways. Some, like PAWLOW and PARASTSCHUK, consider the rennet action simply as the reverse of the synthetical action of pepsin, a view which is improbable in the highest degree. Others, such as SAWJALOW[3] and GEWIN, consider, on the contrary, that the coagulation of milk is only a pepsin action and indeed as the first step in the beginning of proteolysis, namely the beginning of peptic digestion of casein.

The simultaneous occurrence in the animal and plant kingdoms of enzymes having a proteolytic and rennet action indicates an identity of both enzymes and enzyme actions and the parallelism of the pepsin and rennet action. This parallelism in fact does not prove much, because it has mostly been studied in acid reaction, while rennet is characteristically active in neutral or faintly alkaline reaction.

The pathological cases in man, if the observations are reliable, where only one enzyme action occurs, seems to suggest the identity of the action of both enzymes. In opposition, however, is the fact that pepsin, so far as known, only has a digestive action in the presence of free H ions, while the coagulation of milk occurs in the absence of these and indeed in the presence of HO ions. Among other facts which contravene the identity is the fact that a pepsin solution can be prepared which has a digestive action but cannot coagulate milk, and the reverse, namely, rennet solutions can be made which coagulate milk but do not have digestive action in acid reaction (HAMMARSTEN). The observations of

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[1] Hofmeister's Beiträge 2; Bang, l. c.

[2] Zeitschr. f. physiol. Chem., 54.

[3] The recent literature on this question can be found in Hammarsten, Zeitschr. f. physiol. Chem., 56.

Ducceschi,[1] that pepsin but no chymosin occurs in the stomach of the Didelphys, also conflict with the identity of the two enzymes.

The views of Nencki and Sieber[2] take a certain reconciliary position. According to them pepsin forms a gigantic molecule which has various side-chains, one of which has digestive action in acid solution while the others coagulate milk. This view coincides well with most of the observations made thus far.

In regard to the preparation of chymosin solutions free from pepsin and of pepsin solution free from chymosin see the work of Hammarsten.[3]

Plastein. As mentioned on page 134, Danilewsky first showed the power of rennin solutions to cause a partial coagulation of proteoses and of converting them into so-called plastein. It is unknown whether this action is due to pepsin, chymosin or to another enzyme.

Gastric Lipase (stomach steapsin). F. Volhard[4] made the discovery that the gastric juice has a strong fat-splitting action only when the fat is in a fine emulsion, as in the yolk of the egg, in milk or in cream. Considerable controversy has arisen in regard to the importance of the splitting of fat, and the occurrence of a special gastric lipase is indeed disputed. From numerous observations it follows without question that in man and many animals a gastric lipase occurs and is secreted with the gastric juice. Nevertheless the extent of fat splitting in the stomach is generally not very great. In its action this lipase follows Schütz's rule and in its other properties it seems to vary in different animals.

The question whether the cover cells principally or the chief cells also, or both, take part in the formation of free acid is disputed.[5] There can be no doubt that the hydrochloric acid of the gastric juice originates in the chlorides of the blood, because, as is well known, a secretion of perfectly typical gastric juice takes place in the stomachs of fasting animals or those which have starved for some time. As the chlorides of the blood are derived from the food, it is easily understood, as shown by Cahn,[6] that in dogs after a sufficiently long common-salt starvation

[1] Centrabl. f. Physiol., **22**, 784.

[2] Zeitschr. f. physiol. Chem., **32**.

[3] Zeitschr. f. physiol. Chem., **56**.

[4] Volhard, Münch. med. Wochenschr., 1900, and Zeitschr. f. klin. Med., **42**, **43**. See also Stade, Hofmeister's Beiträge, **3**; A. Fromme, *ibid.*, **7**; A. Zinsser, *ibid.*; H. Engel, *ibid.*; and Inouye, Arch. f. Verdauungskrank., **9**; Falloise, Arch. internat. d. Physiol., **3** and **4**; London, Zeitschr. f. physiol. Chem., **50**; Levites, *ibid.*, **49**; Laqueur, Hofmeister's Beiträge, 8, 281; Heinsheimer, Deutsch. med. Wochenschr., **32**, and Arbeiten aus d. pathol. Institute, Berlin (Hirschwald, 1906).

[5] See Heidenhain, Pflüger's Arch., **18** and **19**, and Hermann's Handbuch, **5**, part I, "Absonderungsvorgänge"; Klemensiewicz, Wien. Sitzungsber., **71**; Fränkel, Pflüger's Arch., **48** and **50**; Contejean, l. c.; Kranenburg, Archives Teyler, Ser. II, Haarlem, 1901; and Mo.se, Centralbl. f. Physiol., **17**, 217.

[6] Zeitschr. f. physiol. Chem., **10**.

the stomach secreted a gastric juice containing pepsin, but no free hydrochloric acid. On the administration of soluble chlorides, a gastric juice containing hydrochloric acid was immediately secreted. The conditions are not so simple, because in the first case not only does the amount of hydrochloric acid diminish but, as shown by WOHLGEMUTH, the quantity of juice diminishes greatly, and on the introduction of NaCl the quantity of juice secreted increases. According to PUGLIESE [1] the gastric juice in starvation, after a certain time, has a neutral reaction, and the introduction of NaCl does not now change its properties. In the secretion of free acid it is assumed by PUGLIESE that the gland cells, which decompose the chloride, have sufficient amounts of protein at their disposal. On the introduction of alkali iodides or bromides, KÜLZ, NENCKI and SCHOUMOW-SIMANOWSKI [2] have shown that the hydrochloric acid of the gastric juice is replaced by HBr, and to a less extent by HI. According to KOEPPE the seat of formation of hydrochloric acid is not the blood or the glands, but the interior of the stomach in the immediate neighborhood of the wall. The hydrochloric acid is assumed to be produced from the chlorides of the food, as the semipermeable wall is not permeable for the Cl ions, but is for the Na ions and for the H ions. As the Na ions leave the stomach contents, an equivalent quantity of H ions wander from the blood through the stomach wall into the interior of the stomach and combine with the Cl ions. This theory is difficult to reconcile with the fact that in dogs with sham feeding the empty stomach secretes abundant juice. Other objections have also been raised against this by BENRATH and SACHS. The secretion of free hydrochloric acid from the alkaline blood has been explained in other ways (MALY, BUNGE, L, SCHWARZ), but as yet no satisfactory theory has been suggested.[3]

In regard to the secretion of pepsin we must recall that this last is not already produced, but is formed from a preliminary step, a *pepsinogen* or *propepsin*. LANGLEY [4] has positively shown the existence of such a substance in the mucous coat. This substance, propepsin, shows a comparatively strong resistance to dilute alkalies (a soda solution of 5 p. m.), which easily destroy pepsin (LANGLEY). Pepsin, on the other hand, withstands better than propepsin the action of carbon dioxide, which quickly destroys the latter. The occurrence of a rennin zymogen

[1] Wohlgemuth, Arbeiten aus d. pathol. Institute, Berlin, 1906; Pugliese, Maly's Jahresb., **36**, 394.

[2] Külz, Zeitschr. f. Biologie, **23**; Nencki and Schoumow, Arch. des sciences biol. de St. Pétersbourg, **3**.

[3] Koeppe, Pflüger's Arch., **62**; Benrath and Sachs, *ibid.*, **109**; Maly, see v. Bunge's Lehrbuch der physiol. u. pathol. Chem., 4. Aufl., 1898; Schwarz, Hofmeister's Beiträge, **5**.

[4] Schiff, Leçons sur la physiol. de la digéstion, 1867, **2**; Langley and Edkins, Journ. of Physiol., **7**.

and possibly also of a steapsinogen, in the mucous coat has been mentioned above.

According to HERZEN and his collaborators [1] we must differentiate between pepsinogenic and other bodies accelerating the flow of juice. To the first belong inulin and glycogen, while alcohol belongs to the latter class of bodies. Dextrin not only accelerates the flow of juice, but also acts as a pepsinogenic, especially as the latter. Meat extract which has both actions is especially a flow-accelerator. The pepsinogenic action consists in converting the zymogen into pepsin, and in this way increases the quantity of pepsin; the flow-accelerating substances increase the quantity of secreted juice.

The question in what cells the two zymogens, especially the propepsin, are produced, has been extensively discussed for several years. Formerly it was the general opinion that the cover cells were pepsin cells, but since the investigations of HEIDENHAIN and his pupils, LANGLEY and others, the formation of pepsin has been attributed to the chief cells.[2]

The Pyloric Secretion. That part of the pyloric end of the dog's stomach which contains no fundus glands was dissected by KLEMENSIEWICZ, one end being sewed together in the shape of a blind sac and the other sewed into the stomach. From the fistula thus created he was able to obtain the pyloric secretion of a living animal. This secretion is alkaline, viscous, jelly-like, rich in mucin, of a specific gravity of 1.009–1.010, and containing 16.5–20.5 p. m. solids. It habitually contains pepsin, which has been proven by HEIDENHAIN by observations on a permanent pyloric fistula, and the amount may sometimes be considerable. CONTEJEAN investigated the pyloric secretion in other ways, and finds that it contains both acid and pepsin. The alkaline reaction of the secretions investigated by HEIDENHAIN and KLEMENSIEWICZ is due, according to CONTEJEAN, to an abnormal secretion caused by the operation, because the stomach readily yields an alklaline juice instead of an acid one under abnormal conditions. The reports of HEIDENHAIN and KLEMENSIEWICZ have nevertheless been substantiated by ÅKERMANN, KRESTEFF, SCHEMIAKINE and others.[3]

The secretion of gastric juice under different conditions may vary considerably. The statements concerning the quantity of gastric juice secreted in a certain time are therefore unreliable. ROSEMANN [4] observed on sham feeding in dogs a secretion of 917 cc. in the course of 3½ hours—a considerable quantity.

The Chyme and the Digestion in the Stomach. By means of the chemical stimulation caused by the food, a copious secretion of gastric

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Pflüger's Arch., 84.

See footnote 5, p. 452.

Heidenhain and Klemensiewicz, l. c.; Contejean, l. c., Chapter II, and Skand. Arch f. Physiol, 6, Akermann, *ibid.*, 5; Kresteff, Maly's Jahresber., 30; Schemiakine Arch. des scienc. biolog. de St. Pétersbourg, 10.

Pflüger's Arch., 118.

juice occurs, which gradually mixes with the swallowed food, and digests it more or less strongly. The material in the stomach during digestion, which has a pasty or thick consistency and is called chyme, is not a homogeneous mixture of the ingesta with the various digestive fluids, gastric juice, saliva, and gastric mucus, but the conditions seem to be more complicated.

From the investigations of several workers, such as HOFMEISTER and SCHÜTZ, MORITZ, CANNON, SCHEMIAKINE,[1] and others, on the movements of the stomach, we conclude that this organ in carnivora and also in man consists of two physiologically different parts, the pylorus and the fundus. The greater fundus part, which serves essentially as a reservoir, may by a rhythmic, strong contraction of the muscle, acting like a sphincter between it and the pylorus part, be separated from the latter, and according to some observers so completely so that during contraction scarcely anything passes from the fundus to the pylorus part. Differing from the fundus part, the pylorus is the seat of very powerful contractions by which its contents are intimately mixed with gastric juice and are also driven through the pyloric valve into the intestine.

The contents of the pylorus part have an acid reaction, and a strong pepsin digestion takes place in the contents, which are thoroughly mixed with gastric juice. The contents of the fundus, on the contrary, show a different behavior, for here, as ELLENBERGER first showed, a special stratification of the various solid food-stuffs takes place.

By very instructive investigations on different animals (frogs, rats, rabbits, guinea-pigs, and dogs) GRÜTZNER[2] later showed that when the animals are fed with food having different colors, and the stomach removed after a certain time, and the contents frozen, the frozen sections show a regular stratification of the contents. These layers are so arranged that the food first taken is found in direct contact with the mucosa, while the food taken later is inclosed by that partaken of first, and this prevents contact with the walls of the stomach. The empty stomach, whose walls touch each other, is so filled that, as a rule, the foodstuffs taken later are in the middle of the older food.

Because of this fact only the foodstuffs which lie close to the surface of the mucous membrane undergo the process of peptic digestion, and it is principally these ingesta, which lie on the surface and are laden with pepsin and mixed with gastric juice, which are shoved to the pylorus end, here mixed and digested, and finally moved into the intestine. The fundus part is therefore less a digestion-organ than a storage-organ, and

[1] Hofmeister and Schütz, Arch. f. exp. Path. u. Pharm., **20**; Moritz, Zeitschr. f. Biologie, **32**; Cannon, Amer. Journ. of Physiol., **1**; Schemiakine, l. c.

[2] See Ellenberger, Pflüger's Arch., **114**, and Scheunert, *ibid.*, **144**; Grützner, *ibid.*, **106**.

in the interior of the same the food may remain for hours without coming in contact with a trace of gastric juice.

What has been said above applies at least to solid food. We have no extensive observations on the behavior of fluids or semifluid food. According to GRÜTZNER, in these cases, as well as in the above-mentioned experiments, the swallowed foodstuffs are not irregularly mixed together. Fluids quickly leave the stomach, which is also the case with a mixture of solid and fluid food.

The fact that only that part of the ingesta lying on the mucous membrane is mixed with gastric juice, while the mass in the interior is not acid in reaction, is of special importance for the digestion of starches in the stomach. By this we can explain why the salivary diastase, although sensitive toward acids, can continue its action for a long time in the contents of the stomach. That this is true was first found by ELLENBERGER and HOFMEISTER and then by CANNON and DAY[1] by special experiments upon animals. The occurrence of sugar and dextrin in the contents of the human stomach has been repeatedly observed. In carnivora, whose saliva shows scarcely any diastatic action, it is *a priori* not expected that there should be a diastatic action in the stomach, but the conditions are different in herbivora, where an abundant digestion of starch takes place in the various stomachs, due to the different species.

The gastric contents which have been prepared in the pylorus part are passed through the pylorus into the intestine intermittently. This material is generally fluid, but it is possible that pieces of solid food may also occur, and this has often been observed. Thin or plastic food leaves the stomach earlier than solid food, and it is obvious that the time in which the stomach unburdens itself depends naturally upon the coarseness or fineness of the food. This depends essentially upon the reflex action of the stomach or intestine, causing an opening or closing of the pylorus, which action is dependent upon the quantity and character of the food, the amount of fat, and the degree of acidity in the contents of the stomach and intestine. The emptying of the food into the small intestine causes, as shown by PAWLOW, a closing of the pylorus by chemo-reflex in which the hydrochloric acid and the fat take part, and we thus find in this regard an alternate action between the stomach and duodenum.

This alternate action, according to CANNON[2] is due to the fact that the acid in the pylorus which acts upon the sphincter and makes possible the passage of the fluid chyme by the contraction of the muscles of the stomach. In the intestine the acid has a reverse stimulation upon the sphincter and causes a contraction of the same. As soon as the acid

[1] Ellenberger and Hofmeister, Maly's Jahresb., 15 and 16; Cannon and Day, Amer. Journ. of Physiol., 9.

[2] Amer. Journ. of Physiol., 20.

is neutralized the contractions of the sphincter cease and the passage of new portions of the chyme occur. If the flow of bile and pancreatic juice is prevented and the neutralization of the acid contents of the stomach in the intestine is retarded, then the stomach does not eject its contents so often. The duration of gastric digestion varies according to conditions, and in consequence the reports of observers are widely divergent. BEAUMONT [1] found in his extensive observations on the Canadian hunter St. MARTIN that the stomach, as a rule, is emptied 1½–5½ hours after a meal, depending upon the character of the food.

The time in which different foods leave the stomach also depends upon their digestibility. Respecting the unequal digestibility in the stomach we must differentiate between the rapidity with which the foodstuffs are chemically transformed and that with which they leave the stomach and pass into the intestine. This distinction is especially important, and it is evident that the main factors governing speed of digestion and the time required before the food leaves the stomach are the kind of food and the fineness of its subdivision, and its action upon the gastric secretion, upon the pyloric reflexes, etc.

The observations of BOLDYREFF [2] on the action of fats are conclusive concerning the manner in which the properties of the food act upon the gastric secretion and upon the digestion in the stomach as a whole. Irrespective of the reducing action of the fats upon the extent and digestive power of the gastric juice BOLDYREFF found after food very rich in fat that the bile, pancreatic juice and intestinal juice migrate from the intestine into the stomach so that the digestion in the stomach in these cases is essentially brought about by the pancreatic juice.

We have numerous investigations on the rapidity with which the food is digested in the stomach of dogs, but we must especially mention the researches of E. ZUNZ,[3] LONDON [4] and his co-workers. LONDON, POLOWZOWA and SAGELMANN [5] have observed that all the foodstuffs do not leave the stomach with the same rapidity, indeed, by feeding with bread (POLOWZOWA) the carbohydrates leave more quickly than the protein, and with a mixture of gliadin and beef-fat (SAGELMANN) the protein left the stomach more quickly than the fat. According to these

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[1] The Physiology of Digestion, 1833.

[2] Pflüger's Arch., **121**. See also Abderhalden and Medigreceanu, Zeitschr. f. physiol. Chem., **57**.

[3] E. Zunz, Hofmeister's Beiträge, **3**; Annal de la soc. roy. des sceinc. med. Bruxelles, **12**, **13**· and Mémoires publ. par l'Acad. roy. Belg., 1906, 1907, and 1908.

[4] The numerous works of London and co-workers will be found in Zeitschr. f. physiol. Chem., **45**–**53**, **55**–**57**.

[5] London with Polowzowa, Zeitschr. f. physiol. Chem., **49**, with Sagelmann, *ibid.*, **52**.

authors the stomach has a sort of "selective capacity," but this is strongly disputed by SCHEUNERT and GRIMMER.[1] Nevertheless the researches of CANNON[2] on cats, making use of another method, have shown that this is true. After preliminary hunger the animals received different food, such as meat, fat, and carbohydrate mixed with bismuth subnitrate and then with the aid of the RÖNTGEN rays the time was noted when the food passed into the intestine. The carbohydrate leaves the stomach first, the proteins next, and the fats last. If the carbohydrate is given before the protein food, then it leaves the stomach with ordinary rapidity; while if protein food and then carbohydrate is given the passage of the carbohydrate is retarded. A mixture of protein food and carbohydrates leaves the stomach more slowly than carbohydrates alone, but faster than protein food alone. The fat, which remains in the stomach for a long time and leaves the stomach only in amounts which are absorbed or removed from the duodenum, retards the passage of the protein foods as well as the carbohydrates.

The reason why different food-stuffs leave the stomach with unequal rapidity is explained by CANNON by the above-mentioned action of the hydrochloric acid upon the pyloric sphincter. The proteins combine with the hydrochloric acid and hence its action upon the sphincter becomes weaker, while this is not the case with the carbohydrates. If the carbohydrates are moistened with alkali they leave the stomach more slowly than usual and the acid proteins, on the contrary, leave the stomach earlier than other proteins.

As our knowledge of the digestibility of the different foods in the stomach is slight and uncertain, so also our knowledge of the action of other bodies, such as alcoholic drinks, bitter principles, spices, etc., on the natural digestion is very uncertain and imperfect. The difficulties which stand in the way of this kind of investigation are very great, and therefore the results obtained thus far are often ambiguous or conflict with each other. For example, certain investigators have observed that small quantities of alcohol or alcoholic drinks do not prevent but rather facilitate digestion; others observed only a disturbing action, while still others report having found that the alcohol first acts somewhat as a disturbing agent, but afterward, when it is absorbed, produces an abundant secretion of gastric juice, and thereby facilitates digestion. The accelerating action of alcohol upon the flow of gastric juice has already been mentioned on page 439.

In regard to the importance of the stomach we used to be of the general opinion that an abundant peptonization of protein does not occur in the stomach, and that the food rich in protein is more likely to be chiefly prepared in the stomach for the real digestion in the intestine. That the

[1] Scheunert, Zeitschr. f. physiol. Chem., 51; Grimmer, Bioch. Zeitschr., 3.
[2] Amer. Journ. of Physiol., 12 and 20.

stomach, at least the fundus, acts in the first place as a storage chamber, follows from the shape of this organ, especially in certain animals, and this function becomes especially prominent in certain new-born animals, as dogs and cats. In these animals the gastric secretion contains acid but no pepsin, and the casein of the milk is precipitated by the acid alone as solid lumps or as a solid coagulum filling the stomach. Gradually small quantities of this coagulum pass into the intestine and an overburdening of the intestine is thus prevented. In other animals, as the snake and certain fishes which swallow entire animals, the major part of the digestive work goes on in the stomach. The importance of the stomach for digestion cannot therefore be established in all instances. It varies in different animals and differs even in individual animals of the same species, depending upon the fineness or coarseness of the food, upon the greater or less rapidity with which peptonization takes place, and also upon the rapid or slow increment in the quantity of hydrochloric acid, etc.

In regard to the extent of chemical digestive work, i.e., in the first place the destruction of protein in the stomach, we have numerous researches, some carried out by the use of older methods and others by using newer and more reliable methods. Among these latter we must mention those of Zunz, London and collaborators, Tobler, Lang and Cohnheim.[1] These investigations refer to the conditions in dogs, and as shown by Rosenfeld[2] in horses, and by Lötsch[3] in pigs, that the conditions are different in other animals. The following description applies only to dogs.

In the dog Abderhalden, London and co-workers[4] have shown that in the stomach proteoses and peptones, besides so-called rest bodies, are formed, but no amino-acids, or at least in any mentionable quantity. In like manner we must agree in the belief that a part of the protein always leaves the stomach undigested and that the chief mass, about 80 per cent, passes into the intestine more or less digested. London and Sandberg[5] indeed have from their experiments with gliadin or with egg albumin (London), suggested the proposition that a certain percentage of the partaken protein is always digested, irre-

[1] Tobler, Zeitschr. f. physiol. Chem., 45; Lang, Bioch. Zeitschr., 2; Cohnheim, Münch. med. Wochenschr., 1907. In regard to the works of Zunz, London, and collaborators, see footnotes 3 and 4, p. 457.

[2] Rosenfeld, Ueber die Eiweissverdauung im Magen des Pferdes, Inaug.-Dissert., Dresden, 1908.

[3] Lötsch, Zur Kenntnis der Verdauung von Fleisch im Magen und Dünndarm des Schweines, Inaug.-Dissert. Freiburg i. Sa., 1908.

[4] Abderhalden and London, with Kautsch, Zeitschr. f. physiol. Chem., 48, with L. Baumann, *ibid.*, 51, and with v. Körösy, *ibid.*, 51.

[5] Zeitschr. f. physiol. Chem., 56

spective of the quantity partaken of. We express this by the formula, $\frac{(q-Fr).100}{q}=K$, where q is the quantity of protein fed, Fr is the undigested part and K a constant. For the division of the digested protein into the three groups—proteoses, peptones and rest bodies, figures have also been given. On comparing the results obtained by different investigators one finds such great discrepancies that no positive conclusions can be drawn. Still it seems to be certain that the rest-bodies occur to a very small extent as compared with the proteoses and peptones. Besides this it also seems as if the peptones occur in the pylorus part to a greater extent than the proteoses, while in the fundus part the reverse is the case. Of the dissolved protein of the entire stomach-contents about 60 per cent exists as proteoses. Opinions are also contradictory in regard to the absorption of the decomposition products of the proteins in the stomach. While several investigators, like Tobler, Lang, Cohnheim, Zunz and others accept such an absorption, London and co-workers positively deny this.

The digestion of sundry foods is not dependent on one organ alone, but is divided among several. For this reason it is to be expected that the various digestive organs can act for one another to a certain extent, and that therefore the work of the stomach could be taken up more or less by the intestine. This in fact is the case. Thus the stomachs of dogs and cats have been completely extirpated or nearly so (Czerny, Carvallo and Pachon), and that part necessary in the digestive process has also been eliminated by plugging the pyloric opening (Ludwig and Ogata), and in both cases it was possible to keep the animal alive, well fed, and strong for a shorter or longer time. This is also true for human beings.[1] In these cases it is evident that the digestive work of the stomach was taken up by the intestine; but all food cannot be digested in these cases to the same extent, and the connective tissue of meat especially is sometimes found to a considerable extent undigested in the excrements.

It is a well-known fact that the contents of the stomach may be kept without decomposing for some time by means of hydrochloric acid, while, on the contrary, when the acid is neutralized a fermentation commences by which lactic acid and other organic acids are formed. According to Cohn an amount of hydrochloric acid above 0.7 p. m. completely arrests lactic-acid fermentation, even under otherwise favorable circumstances, and according to Strauss and Bialocour the limit of lactic-

[1] Czerny, cited from Bunge, Lehrbuch d. physiol. u. path. Chem. 4. Aufl., Theil 2, 173; Carvallo and Pachon, Arch. d. Physiol. (5), 7; Ogata, Arch. f. (Anat. u.) Grohé, Arch. f. exp. Path. u. Pharm. 49; in regard to the case in man, see Schlatter in Wróblewski, Centralbl. f. Physiol. 11, p. 665, and the surgical journals.

acid fermentation lies at 1.2 p. m. hydrochloric acid united to organic bodies. The hydrochloric acid of the gastric juice has unquestionably an antifermentative action, and also, like all dilute mineral acids, an antiseptic action. This action is of importance, as many pathogenic micro-organisms may be destroyed by the gastric juice. The common bacillus of cholera, certain streptococci, etc., are killed by the gastric juice, while others, especially as spores, are unacted upon. The fact that gastric juice can diminish or retard the action of certain toxalbumins, such as tetanotoxine and diphtheria toxine, is also of great interest (NENCKI, SIEBER, and SCHOUMOWA [1]).

Because of this antifermentative and antitoxic action of gastric juice it is considered that the chief importance of the gastric juice lies in its antiseptic action. The fact that intestinal putrefaction is not increased on the extirpation of the stomach, as derived from experiments made on man and animal,[2] does not uphold this view.

Since the hydrochloric acid of the gastric juice prevents the contents of the stomach from fermenting, with the generation of gas, those *gases* which occur in the stomach probably depend, at least in great measure, upon the swallowed air and saliva, and upon those gases generated in the intestine and returned through the pyloric valve. PLANER found in the stomach-gases of a dog 66–68 per cent N, 23–33 per cent CO_2, and only a small quantity, 0.8–6.1 per cent, of oxygen. SCHIERBECK [3] has shown that a part of the carbon dioxide is formed by the mucous membrane of the stomach. The tension of the carbon dioxide in the stomach corresponds, according to him, to 30–40 mm. Hg in the fasting condition. It increases after partaking food, independently of the kind of food, and may rise to 130–140 mm. Hg during digestion. The curve of the carbon-dioxide tension in the stomach is the same as the curve of acidity in the different phases of digestion, and SCHIERBECK also found that the carbon-dioxide tension is considerably increased by pilocarpine, but diminished by nicotine. According to him, the carbon dioxide of the stomach is a product of the activity of the secretory cells.

After death, if the stomach still contains food, autodigestion goes on not only in the stomach, but also in the neighboring organs, during the slow cooling of the body. This leads to the question, Why does the stomach not digest itself during life? Ever since PAVY has shown that

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[1] Cohn, Zeitschr. f. physiol. Chem., **14**; Strauss and Bialocour, Zeitschr. f. klin. Med., **28**. See also Kühne, Lehrb., 57; Bunge, Lehrb. d. Physiol., 4. Aufl., 148 and 159; Hirschfeld, Pfluger's Arch., **47**; Nencki, Sieber, and Schoumowa, Centralbl. f. Bacteriol., etc., **23** In regard to the action of gastric juice upon pathogenic microbes we must refer the reader to handbooks of bacteriology.

[2] See Carvallo and Pachon, l. c., and Schlatter in Wróblewski, l. c.

[3] Planer, Wien. Sitzungsber., **42**; Schierbeck, Skand. Arch. f. Physiol., **3** and **5**.

after tying the smaller blood-vessels of the stomach of dogs the corresponding part of the mucous membrane was digested, efforts have been made to find the cause in the neutralization of the acid of the gastric juice by the alkali of the blood. That the reason for the non-digestion during life is to be sought for in the normal circulation of the blood cannot be contradicted; but the reason is not to be found in the neutralization of the acid. The investigations of FERMI and OTTE [1] show that the blood circulation acts in an indirect manner by the normal nourishment of the cell protoplasm, and this is the reason why the digestive fluids, the gastric juice as well as the pancreatic juice, act differently upon the living protoplasm as compared with the dead. We know nothing about this resistance of the living protoplasm. Some claim that it is closely connected with the secretion of the antipepsins discovered by DANILEWSKY, HÄNSEL, and WEINLAND, but this is hard to understand. Undoubtedly bodies occur in the gastric mucosa which can inhibit the action of pepsin, but whether these bodies are of an enzymotic nature or not is undecided. WEINLAND'S antipepsin is related to the enzymes because it is thermolabile, while the antipepsin of DANILEWSKY, HÄNSEL, and O. SCHWARZ [2] is resistant toward heat and can hardly be considered as an enzymotic body. This is true for at least the thermostabile antipepsin of SCHWARZ, which does not give the biuret reaction. Without mentioning the still unknown nature of these bodies, the natural gastric juice, as well as an acid infusion of the mucosa, has such a strong digestive action that the retarding action of the antipepsin can only be shown under special conditions, and it is therefore difficult to conceive how the antipepsin could have a protective action in life.

Under pathological conditions irregularities in the secretion may occur. The quantity of enzymes may be diminished and both enzymes or, as found in certain cases, one (the chymosin), may be absent. The hydrochloric acid may also be absent or may exist in very small amounts. A pathological high degree of acidity of the pure juice is not very probable, while on the contrary a hypersecretion of gastric juice in different forms does occur.

In testing the gastric juice or the filtered stomach contents, diluted with digestive hydrochloric acid, for *pepsin* we make use of the pepsin tests given on pages 445, 446. In testing for *rennin* the liquid must be first carefully neutralized and 1–2 cc. of this liquid added to 10 cc. milk. In the presence of appreciable quantities of rennin, the milk

[1] Pavy, Phil. Transactions, **153**, part I, and Guy's Hospital Reports, **13**; Otte, Travaux du laboratoire de l'Institut de Physiol. de Liège, **5**, 1896, which also contains the literature.

[2] See Hänsel, Biochem. Centralbl., **1**, p. 404, and **2**, p. 326; Weinland, Zeitschr. f. Biologie, **44**; Schwartz, Hofmeister's Beiträge, **6**.

should coagulate at room temperature within 10–20 minutes without changing its reaction. The addition of lime salts is unnecessary, and may readily lead to erroneous conclusions.

In many cases it is especially important to determine the *degree of acidity of the gastric juice.* This may be done by the ordinary titration methods. Phenolphthalein must not be used as an indicator, as too high results are produced in the presence of large quantities of proteins. Good results may be obtained, on the contrary, by using very delicate litmus paper. Although the acid reaction of the contents of the stomach may be caused simultaneously by several acids, still the degree of acidity is here, as in other cases, expressed in only one acid, e.g., HCl. Generally the acidity is designated by the number of cubic centimeters of N/10 sodium hydroxide required to neutralize the several acids in 100 cc. of the liquid of the stomach. An acidity of 43 per cent means that 100 cc. of the liquid of the stomach required 43 cc. of N/10 sodium hydroxide to neutralize it.

It is also important to be able to ascertain the nature of the acid or acids occurring in the contents of the stomach. For this purpose, and especially for the *detection of free hydrochloric acid,* a great number of color reactions have been proposed which are all based upon the fact that the coloring substance gives a characteristic color with very small quantities of hydrochloric acid, while lactic acid and the other organic acids do not give these colorations, or only in a certain concentration, which can hardly exist in the contents of the stomach. These reagents are a mixture of FERRIC-ACETATE and POTASSIUM-SULPHOCYANIDE solutions (MOHR'S reagent has been modified by several investigators), METHYL-ANILINE-VIOLET, TROPÆOLIN 00, CONGO RED, MALACHITE-GREEN, PHLORO-GLUCINOL-VANILLIN, DIMETHYLAMINOAZOBENZENE, and others. As reagents for *free lactic acid* UFFELMANN suggests a strongly diluted, amethyst-blue solution of FERRIC CHLORIDE and CARBOLIC ACID or a strongly diluted, nearly colorless solution of FERRIC CHLORIDE. These give a yellow color with lactic acid, but not with hydrochloric acid or with volatile fatty acids.

The value of these reagents in testing for free hydrochloric acid or lactic acid is still disputed. Among the reagents for free hydrochloric acid it seems STEENSMA S [1] modification of GÜNZBURG'S test with phloroglucinol-vanillin, and the test with tropæolin 00, performed at a moderate temperature as suggested by BOAS, and the test with dimethylaminoazobenzene, which is the most delicate, seem to be the most valuable. If these tests give positive results, then the presence of hydrochloric acid may be considered as proven. A negative result does not eliminate the presence of hydrochloric acid, as the delicacy of these reactions has a limit, and also the simultaneous presence of protein, peptones, and other bodies influences the reactions more or less. The reactions for lactic acid may also give negative results in the presence of comparatively large quantities of hydrochloric acid in the liquid to be tested. Sugar, sulphocyanides, and other bodies may act with these reagents like lactic acid.

In testing for lactic acid it is safest to shake the material with ether and test the residue after the evaporation of the solvent. On the evaporation of the ether the residue may be tested in several ways. BOAS utilizes the property possessed by lactic acid of being oxidized into aldehyde and formic acid on careful oxidation with sulphuric acid and manganese dioxide. The aldehyde is detected by

[1] Bioch. Zeitschrift, 8.

its forming iodoform with an alkaline iodine solution or by its forming aldehyde-mercury with NESSLER'S reagent. CRONER and CRONHEIM [1] have suggested another method.

The quantitative estimation consists in the formation of iodoform with N/10 iodine solution and caustic potash, adding an excess of hydrochloric acid and titrating with a N/10 sodium-arsenite solution, and retitrating with iodine solution, after the addition of starch-paste, until a blue coloration is obtained. This method presupposes the use of ether entirely free from alcohol. For details see the original publication and the modification of this method suggested by JERUSALEM.[2]

In order to be able to judge correctly of the value of the different reagents for free hydrochloric acid, it is naturally of greatest importance to be clear in regard to what we mean by free hydrochloric acid. It is a well-known fact that hydrochloric acid combines with proteins, and a considerable part of the hydrochloric acid may therefore exist in the contents of the stomach, after a meal rich in proteins, in combination with them. This hydrochloric acid combined with proteins cannot be considered as free, and it is for this reason that certain investigators consider such methods as that of SJÖQVIST, which will be described below, as of little value. However, it must be remarked that, according to the unanimous experience of many investigators, the hydrochloric acid combined with proteins is physiologically active and in this regard we must refer to the recent investigations of ALB. MÜLLER and J. SCHÜTZ.[3] Those reactions (color reactions) which only respond to actually free hydrochloric acid do not show the physiologically active hydrochloric acid. The suggestion of determining the "physiologically active" hydrochloric acid instead of the "free" seems to be correct in principle; and as the conceptions of free and of physiologically active hydrochloric acid are not the same, it must always be well defined whether one wishes to determine the actually free or the physiologically active hydrochloric acid before any conclusions are drawn as to the value of a certain reaction.

The acid reaction may be partly due to free acid, partly to acid salts (monophosphates), and partly to both. According to LEO [4] one can test for acid phosphates by calcium carbonate, which is not neutralized therewith, while the free acids are. If the gastric content has a neutral reaction after shaking with calcium carbonate and the carbon dioxide is driven out by a current of air, it contains only free acid; if it has an acid reaction, acid phosphates are present, and if it is less acid than before, it contains both free acid and acid phosphate. It must not be forgotten that a faint acid reaction may, after treatment with calcium carbonate, also be due to the protein. This method can likewise be applied in the estimation of free acid.

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[1] Beas. Deutsch. med. Wochenschr., 1893, and Münchener med. Wochenschr, 1893, Croner and Cronheim, Berl. klin. Woschenschr., 1905. See also Thomas, Zeitschr. f. physiol. Chem., 50.

[2] Bioch. Zeitschr., 12.

[3] Alb. Müller, Deutsch. Arch. f. klin. Med., 88, and Pflüger's Arch., 116; J. Schütz, Wien. klin. Wochenschr., 20, and Wien. med. Wochenschr., 1906 (older literature).

[4] Centralbl. f. d. med. Wissensch., 1889, p. 481; Pflüger's Arch., 48, and Berlin. klin. Wochenschr., 1905, p. 1491.

Various titration methods have been suggested for the estimation of the free hydrochloric acid, but these cannot yield conclusive results for the reasons given in a previous chapter (see estimation of the alkalinity of the blood-serum, page 264). For this determination physico-chemical methods are necessary, but they have not been used to any great extent for clinical purposes on account of the difficulty in their manipulation.

A great number of methods have been suggested for the quantitative estimation of the total acidity, among which we must mention those of K. MÖRNER and SJÖQUIST, which are extensively used. As the value of a special determination of the free and total hydrochloric acid is doubtful, or at least disputed, and also as the question is chiefly of clinical interest we must refer to the hand-books of clinical investigations of v. JAKSCH, EULENBURG, KOLLE and WEINTRAND and of SAHLI. The same applies to the tests for volatiles fatty acids.

III. THE GLANDS OF THE MUCOUS MEMBRANE OF THE INTESTINE AND THEIR SECRETIONS.

The Secretion of Brunner's Glands. These glands are partly considered as small pancreatic glands and partly as mucous or salivary glands. Their importance is not the same in all animals. According to GRÜTZNER they are in dogs closely related to the pyloric glands and contain pepsin. This also coincides with the observations of GLAESSNER and of PONOMAREW, which differ from each other only in that PONOMAREW finds that the secretion is inactive in alkaline reaction and contains only pepsin, while GLAESSNER claims it is active in both acid and alkaline reaction and that it contains pseudopepsin. According to ABDERHALDEN and RONA the pure duodenal secretion of the dog contains a proteolytic enzyme which does not belong to the trypsin type but rather to the pepsin variety. The statements as to the occurrence of a diastatic enzyme in BRUNNER'S glands are disputed. SCHEUNERT and GRIMMER [1] indeed found diastatic enzyme in the duodenal glands of the horse, ox, pig and rabbit, but no proteolytic or rennin enzyme.

The Secretion of Lieberkuhn's Glands. The secretion of these glands has been studied with the aid of a fistula in the intestine according to the method of THIRY and VELLA or of PAWLOW. According to BOLDYREFF [2], in dogs with an empty stomach a scanty secretion lasting about 15 minutes occurs at regular intervals for about two hours. According to this experimenter, during gastric digestion the juice is periodically but less abundantly secreted as the time interval is much longer, namely three, four to five hours. Otherwise it is generally admitted that the partaking of food causes the secretion, or if this is continuous, as in lambs (PREGL), it increases the secretion. The researches of DELEZENNE and FROUIN

[1] Grützner, Pflüger's Arch., **12**; Glaessner, Hofmeister's Beiträge, **1**; Ponomarew, Biochem. Centralbl., **1**, 351; Abderhalden and Rona, Zeitschr. f. physiol. Chem., **47**; Scheunert and Grimmer, cited in Bioch. Centralbl., **5**, 673.

[2] Thiry, Wien. Sitz.-Ber., **50**; Vella, Molleschott's Untersuch., **13**; Boldyreff, Zeitschr. f. physiol. Chem., **50**.

show without question that the passage of chyme into the intestine increases the secretion of the intestinal juice. The acid causes a formation of secretin (see below), and this produces, according to the above investigators, a secretion of intestinal juice. As the secretin undoubtedly also increases the secretion of pancreatic juice and as this latter, according to PAWLOW, by its action upon the intestine excites a secretion of intestinal juice, it is difficult to understand why, according to BOLDYREFF, the secretion of intestinal juice should be so weak during the entire gastric digestion, sometimes so weak that little if any juice is secreted. Soaps, chloral, ether and on intravenous injection, also intestinal juice or an extract of the intestinal mucosa (FROUIN), are chemical excitants of intestinal juice. Several salts, NaCl, Na_2SO_4, and others, may cause an abundant secretion of fluid into the intestine when injected intravenously or subcutaneously, as well as after direct application to the peritoneal surface of the intestine. This action can be arrested by the antagonistic, inhibiting action of a lime salt (MACCALLUM). Pilocarpine, which has the power of increasing the activity of secretions, does not increase the secretion in lambs, and in dogs it does not seem to be always active (GAMGEE [1]).

Mechanical irritation of the intestinal mucosa increases the secretion in dogs (THIRY) as well as in man (HAMBURGER and HEKMA), but it is still doubtful whether we here have a perfectly physiological juice. In the cases observed by HAMBURGER and HEKMA [2] the flow of fluid was greatest at night as well as between five and eight o'clock in the afternoon, and was lowest between two and five o'clock in the afternoon. The quantity of this secretion in the course of twenty-four hours has not been exactly determined.

According to DELEZENNE and FROUIN, if any mechanical irritation is prevented, the fluid flowing spontaneously from a fistula in a dog is ten times more abundant in the duodenum than that in the middle or lower part of the jejunum. In the upper part of the small intestine of the dog, on the contrary, this secretion is scanty, slimy, and gelatinous; in the lower part it is more fluid, with gelatinous lumps or flakes (RÖHMANN). Intestinal juice has a strong alkaline reaction toward litmus, generates carbon dioxide on the addition of an acid, and contains (in dogs) nearly a constant quantity of NaCl and Na_2CO_3, 4.8–5 and 4–5 p. m. respectively (GUMILEWSKI, RÖHMANN [3]). The intestinal juice of the lamb corresponded to an alkalinity of 4.54 p. m. Na_2CO_3. It

[1] Delezenne and Frouin, Compt. rend. soc. biol., **56**; Frouin, *ibid.*, **56** and **58**; MacCallum, University of California Publications, **1**, 1904; Gamgee, Physiol. Chemistry, **2**, 410 (literature).

[2] Journ. de Physiol. et d. path. gén., 1902 and 1904.

[3] Gumilewski, Pflüger's Arch., **39**, Röhmann, *ibid.*, **41**.

contains protein (THIRY found 8.01 p. m.), the quantity decreasing with the duration of the elimination. The quantity of solids varies. In dogs the quantity of solids is 12.2–24.1 p. m. and in lambs 29.85 p. m. The specific gravity of the intestinal juice of the dog, according to the observations of THIRY, is 1.010–1.0107, and in lambs 1.0143 (PREGL). The intestinal juice from lambs contains 18.097 p. m. protein, 1.274 p. m. proteoses and mucin, 2.29 p. m. urea, and 3.13 p. m. remaining organic bodies.

We have the investigations of DEMANT, TURBY and MANNING, H. HAMBURGER and HEKMA and NAGANO [1] on the human intestinal juice. Human intestinal juice has a low specific gravity, nearly 1.007, about 10–14 p. m. solids, and is strongly alkaline toward litmus. The content of alkali calculated as sodium carbonate is 2.2 p. m., according to NAGANO, HAMBURGER and HEKMA, and 5.8–6.7 p. m. NaCl. The determination of the freezing-point was –0.62° (HAMBURGER and HEKMA).

The intestinal juice of the dog contains, according to BOLDYREFF,[2] a *lipase* which acts especially upon emulsified fat (milk), and is different from pancreas lipase, in that its action is not accelerated by bile. The intestinal juice of animals and man also contains an enzyme, *erepsin*, discovered by O. COHNHEIM, which does not ordinarily have a splitting action upon native proteins, but upon proteoses and peptones. It also possibly contains a *nuclease*, and it also has a faint *amylolytic action*. The juice, and to a high degree the mucous coat, contains *invertase* and *maltase*, which fact has been substantiated by the observations of PASCHUTIN, BROWN and HERON, BASTIANELLI, and TEBB.[3] A lactose-inverting enzyme, a *lactase*, also occurs, as shown by RÖHMANN and LAPPE, PAUTZ and VOGEL, WEINLAND, and ORBAN,[4] in new-born infants and young animals, and also in grown mammals which were fed upon a milk diet. The lactase can be obtained more abundantly from the mucosa than from the juice and according to some occurs only in the cells. The claims as to the occurrence of a glucoside splitting enzyme are disputed (FROUIN, OMI [5]).

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[1] Demant, Virchow's Arch., **75**; Turby and Manning, Centralbl. f. d. med. Wissenschaft, 1892, 945; Hamburger and Hekma, l. c.; Nagano, Mitt. aus d. Grenzgeb. d. Med. u. Chir., **9**.

[2] Boldyreff, Archiv d. sciences biolog. de St. Pétersbourg, **11**.

[3] Paschutin, Centralbl. f. d. med. Wissensch., 1870, 561; Brown and Heron, Annal. d. Chem. u. Pharm., **204**; Bastianelli, Moleschott's Untersuch. zur Naturlehre, **14** (this contains all the older literature). See also Miura, Zeitschr. f. Biologie, **32**; Widdicombe, Journ. of Physiol., **28**; Tebb, *ibid.*, **15**.

[4] Röhmann and Lappe, Ber. d. deutsch. chem. Gesellsch., **28**; Pautz and Vogel, Zeitschr. f. Biologie, **32**; Weinland, *ibid.*, **38**; Orban, Maly's Jahresber., **29**.

[5] Frouin and Thomas, Arch. internat. de Physiol., **7**; Omi, Das Verhalten des Salizins im tierischen Organismus. Inaug.-Dissert. Breslau, 1907.

Besides erepsin and the other enzymes mentioned, the intestinal mucosa also contains antienzymes, *antipepsin* and *antitrypsin* (DANILEWSKY and WEINLAND [1]), also *enterokinase* or a mother-substance of the same, and finally also the so-called *prosecretin*. These two last-mentioned bodies, which are closely connected with the secretion of pancreatic juice, will be discussed in connection with this digestive fluid.

The various enzymes are not formed in equal quantities in all parts of the intestine. Diastase and invertase occur, according to BOLDYREFF, all through the intestine, while the lipase on the contrary occurs only in the lower parts. The kinase occurs only in the upper part of the intestine (BOLDYREFF, BAYLISS and STARLING, DELEZENNF). According to HEKMA the kinase occurs in all parts of the intestine, but most abundantly in the duodenum and the upper part of the jejunum. The enzymes, FALLOISE claims, generally occur in greatest abundance in the upper parts of the intestine; but the erepsin occurs to a greater extent in the jejunum than in the duodenum. According to the investigations of VERNON the behavior of erepsin is not the same in different animals. In cats and hedge-hogs the duodenum is richer in erepsin than the jejunum and ileum; in rabbits it is the reverse, namely, the ileum is much richer than the duodenum. The secretin, according to BAYLISS and STARLING, is formed entirely in the upper part of the intestine. The epithelium-cells of the glands or the mucous membrane are generally considered as the seat of formation of the enzymes, and the same is true also for the enterokinase, according to BAYLISS and STARLING, HEKMA, FALLOISE, and others, which, however, DELEZENNE says,[2] is formed in the leucocytes and PEYER's glands.

BOTTAZZI [3] obtained a very complex protein from the intestinal mucosa, which is readily soluble in water and alkali but is precipitated by acids. It coagulates at 55° to 56° and probably also contains carbohydrate and considerable iron. Intravenous injection of this protein brings about an abundant secretion of saliva, pancreatic juice, bile, and intestinal juice, and promotes the peristalic movements of the intestine.

Erepsin. This enzyme, discovered by O. COHNHEIM, has no direct action upon native proteins with the exception of casein, but has the power of splitting proteoses, peptones and certain polypeptides. In this change mono- as well as diamino-acids are produced. Erepsin occurs in the mucous membrane and in the intestinal juice of man as well as of dogs; the mucous membrane seems to be richer than the juice

[1] See footnote 2, p. 462.

[2] Boldyreff, Arch. d. scienc. biolog. de St. Pétersbourg, **11**; Bayliss and Starling, Journ. of Physiol., **29**, **30**; Hekma, l. c.; Falloise, see Biochem. Centralbl., **4**, p. 153; Vernon, Journ. of Physiol., **33**; Delezenne, Compt. rend. soc. biolog., **54** and **56**.

[3] See Biochem. Centralbl., **3**, p. 65.

(SALASKIN, KUTSCHER and SEEMANN[1]). An enzyme like erepsin also occurs in the pancreas (BAYLISS and STARLING, VERNON), and this has the power of acting upon casein, but not, or only faintly, upon fresh fibrin. This erepsin is probably identical with the enzyme *nuclease*, discovered by F. SACHS in the pancreas, which acts upon nucleic acids, while NAKAYAMA claims that erepsin differs from trypsin in having a cleavage action upon nucleic acids. Erepsin shows a great similarity to the intracellular enzymes active in autolysis, and according to VERNON, erepsins occur in the various tissues of invertebrates as well as vertebrates. These tissue erepsins, which are closely related to the autolytic enzymes, if they are not identical with them, behave somewhat differently from the intestinal erepsin and are not identical therewith. Enzymes, having an action similar to erepsin, occur, VINES believes,[2] in all plants so far investigated.

Erepsin becomes inactive on heating to 59°. It works best in alkaline solution, but has hardly any action in faint acid reaction. In this regard, as well as by the fact that only a little ammonia is split off by its action upon peptone substances, it differentiates itself from certain of the autolytic enzymes studied so far. The optimum of alkalinity is, according to EULER,[3] at least in the splitting of a polypeptide, much lower than the optimum for tryptic digestion.

The secretion of the glands in the large intestine seems to consist chiefly of mucus. Fistulas have also been introduced into these parts of the intestine, which are chiefly, if not entirely, to be considered as absorption organs. The investigations on the action of this secretion on nutritive bodies have not as yet yielded any positive results.

IV. THE PANCREAS AND PANCREATIC JUICE.

In invertebrates, which have no pepsin digestion and which also have no formation of bile, the pancreas, or at least an analogous organ, seems to be the essential digestive gland. On the contrary, an anatomically characteristic pancreas is absent in certain vertebrates and in certain fishes. Those functions which should be regulated by this organ seem to be performed in these animals by the liver, which may be rightly called the HEPATOPANCREAS. In man and in most vertebrates the formation of bile, and of certain secretions, containing enzymes important for digestion, is divided between the two organs, the liver and the pancreas.

[1] Cohnheim, Zeitschr. f. physiol. Chem., **33**, **35**, **36**, and **47**; Salaskin, *ibid.*, **35**; Kutscher and Seemann, *ibid.*, **35**.

[2] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30** and **33**; F. Sachs, Zeitschr. f. physiol. Chem., **46**; Nakayama, *ibid.*, **41**; Vines, Annals of Botany, **18**, **19**. and **23**

[3] Zeitschr. f. physiol. Chem., **51**.

The **pancreatic gland** is similar in certain respects to the parotid gland. The secreting elements of the former consist of nucleated cells whose basis forms a mass rich in proteins, which expands in water and in which two distinct zones exist. The outer zone is more homogeneous, the inner cloudy, due to a quantity of granules. The nucleus lies about midway between the two zones, but this position may change with the varying relative size of the two zones. According to HEIDENHAIN [1] the inner part of the cells diminishes in size during the first stages of digestion, in which the secretion is active, while at the same time the outer zone enlarges owing to the absorption of new material. In the later stage, when the secretion has decreased and the absorption of the nutritive bodies has taken place, the inner zone enlarges at the expense of the outer, the substance of the latter having been converted into that of the former. Under physiological conditions the glandular cells are undergoing a constant change, at one time consuming from the inner part and at another time growing from the outer part. The inner granular zone is converted into the secretion, and the outer, more homogeneous zone, which contains the repairing material, is then converted into the granular substance. The so-called islands of LANGERHANS are related to the internal secretion or contain a substance taking part in the transformation of the sugar of the animal body.[2]

The chief portion of protein substances contained in the gland consists, it seems, of a *protein insoluble* in water or neutral salt solution and of *nucleoproteins*, while the *globulin* and *albumin* occur only to a slight extent as compared with the nucleoproteins. Among the compound proteins is the substance studied and isolated by UMBER but previously discovered by HAMMARSTEN [3] and called α-proteid. This nucleoprotein contains, as an average, 1.67 per cent P, 1.29 per cent S, 17.12 per cent N, and 0.13 per cent Fe. It yields β-proteid on boiling, which is much richer in phosphorus than the nucleoprotein. The native proteid (α) is the mother-substance of guanylic acid; according to UMBER it dissolves on pepsin digestion without leaving any residue, and yields on trypsin digestion guanylic acid on one side and proteoses and peptones on the other. It can be extracted from the gland by a physiological salt solution, and is precipitated by acetic acid. Besides this compound protein the pancreas must contain at least one other protein which is the mother-substance of the thymonucleic acid obtainable from the pancreas.

Besides these protein substances the gland also contains several enzymes, or more correctly *zymogens*, which will be discussed later.

[1] Pflüger's Arch., **10**.

[2] See Diamare and Kuliabko, Centralbl. f. Physiol., **18** and **19**; Rennie, *ibid.*, **18**; Sauerbeck, Virchow's Arch., **177**, Suppl.

[3] Umber, Zeitschr. f. klin. Med., **40** and **43**; Hammarsten, Zeitschr. f. physiol. Chem., **19**.

Among the extractive bodies, which are probably in part formed by post-mortem changes and chemical action, we must mention *leucine* (butalanine), *tyrosine, purine bases* in variable quantities,[1] *inosite, lactic acid, volatile fatty acids* and *fats.* The mineral bodies vary considerably in quantity, not only in animals and man but also in men and women (GOSSMANN). The calcium seems, according to GOSSMANN, to exist in much greater amount than the magnesium. According to the investigations of OIDTMANN the pancreas of an old woman contains 745.3 p. m. water, 245.7 p. m. organic and 9.5 p. m. inorganic substances. GOSSMANN [2] found in a man 17.92 p. m. ash and 13.05 p. m. in a woman.

Besides the already-mentioned (Chapter VIII) relation to the transformation of sugar in the animal body, the pancreas has the property of secreting a juice especially important in digestion.

Pancreatic Juice. This secretion may be obtained by adjusting a fistula in the excretory duct, according to the methods suggested by BERNARD, LUDWIG, and HEIDENHAIN, and perfected by PAWLOW.[3]

In herbivora, such as rabbits, whose digestion is uninterrupted, the secretion of the pancreatic juice is continuous. In carnivora, it seems, on the contrary, to be intermittent and dependent on the digestion. During starvation the secretion almost stops, but commences again after partaking of food and reaches its maximum, it is claimed by BERNSTEIN, HEIDENHAIN, and others, within the first three hours. According to PAWLOW and his school (WALTHER [4]) this maximum is dependent upon the character of the food. With milk diet it appears within three to four hours, after bread diet at the end of the second hour, and with a meat diet it arrives still sooner. The quality of the juice is also, according to PAWLOW'S school, dependent upon the food, and the amount of the three enzymes, diastase, trypsin, and steapsin, changes with the variety of food. That the juice is secreted in varying amounts and composition after various foods has been shown by many observers. On the other hand its composition can undergo striking variation with one and the same food (MAZURKIEWICZ), and it is difficult to find any positive connection between the food and the composition of the juice. In man WOHLGEMUTH observed a more abundant secretion after carbohydrates than after food rich in protein. GLAESSNER and POPPER [5]

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[1] See Kossel, Zeitschr. f. physiol. Chem., 8.

[2] Gossmann, Maly's Jahresber., **30**; Oidtmann, cited from Gorup-Besanez, Lehrbuch, 4th ed., 732.

[3] Bernard, Leçons de Physiol., **2**, 190; Ludwig, see Bernstein, Arbeiten a. d. physiol. Anstalt zu Leipzig, 1869; Heidenhain, Pflüger's Arch., **10**, 604; Pawlow, Die Arbeit der Verdauungsdrüsen, Wiesbaden, 1898, and Ergebnisse der Physiologie, **1**, Abt. 1.

[4] Bernstein, l. c., footnote 3, Walther, Arch. des sciences biol. de St. Pétersbourg, **7**.

[5] Mazurkiewicz, Pflüger's Arch., **121**; Wohlgemuth, Berl. klin. Wochenschr., **44**; K. Glaessner and H. Popper, Deutsch. Arch. f. klin. Med., **94**.

observed the reverse in man, that is, after carbohydrate feeding the smallest amount of juice was secreted, and with a mixed diet about the same amount as after the partaking of protein or fat. Objections have also been raised in many quarters against PAWLOW's teachings as to an accommodation of the quantity of enzyme to the kind of food, and some of the observations on which these teachings are based have been somewhat differently explained in the light of recent investigations on the conditions necessary for the activation of the trypsinogen.

PAWLOW and his pupils, especially SCHEPOWALNIKOFF, have shown that the above-mentioned (page 468) enterokinase activates the trypsinogen into trypsin. These observations were later confirmed by others, by DELEZENNE and FROUIN, POPIELSKI, CAMUS and GLEY, BAYLISS and STARLING, ZUNZ, and have been further studied. The pure juice contains, at least as a rule, only trypsinogen, and no trypsin. By mixing with the intestinal juice, or by contact with the intestinal mucosa, the trypsinogen is converted into trypsin by the kinase. Enterokinase, which itself has no action upon proteins, and therefore is not a proteolytic enzyme, is not well known. It is made inactive by heating and is therefore considered by many (including PAWLOW) as an enzyme. Others, on the contrary, like HAMBURGER and HEKMA, DASTRE and STASSANO, deny the enzyme nature of enterokinase because they find that a certain quantity of intestinal juice will activate only a certain quantity of trypsin. Enterokinase has been found in man and all mammals investigated. According to most investigators it is formed in the glands or the cells of the intestinal mucosa, while according to DELEZENNE it comes from PEYER's patches and from the lymph-glands and leucocytes, hence impure fibrin containing leucocytes acts as a kinase. These deductions of DELEZENNE are disputed by BAYLISS and STARLING, HEKMA and others.

From the experience with enterokinase, the report as to the accommodation of the enzyme content of the juice to the kind of food has also been explained by the fact that after different foods the proteolytic power of the juice becomes unequally activated (LINTWAREW and others). For instance, a diet of bread and milk causes the secretion of a large quantity of juice which is rich in trypsinogen but contains almost no trypsin, while after a rich meat diet the secretion becomes scant and the juice contains only trypsin but no trypsinogen. The theory based upon the investigations of DELEZENNE, FROUIN, POPIELSKI, BAYLISS and STARLING, PRYM, and others,[1] that the pure juice collected under the influence of the food contained only trypsinogen but no trypsin, opposes this

[1] In regard to the literature on enterokinase, secretin, and secretion of pancreatic juice, see O. Cohnheim, Biochem. Centralbl., **1**, 169, and S. Rosenberg, *ibid.*, **2**, 708; Prym, Pflüger's Arch., **104** and **107**.

assumption. According to FROUIN [1] the influence of the various foods consists in that the latter give to the juice a different activation. According to FROUIN, for the activation to maximum amount of trypsin the meat juice requires 1/500–1/1000 of its volume of intestinal juice, and bread-juice 1/20–1/10 of its volume. The question as to the influence of the food upon the properties of the juice is still unsettled and requires further study.

If we accept the view that the juice secreted after partaking food is regularly free from trypsin, still under other circumstances the juice may contain trypsin. Thus according to CAMUS and GLEY the juice secreted under the influence of secretin (see below) is not always free from trypsin, and ZUNZ found that WITTE's peptone or pilocarpine causes a secretion of juice which often contained trypsin and was directly active. According to CAMUS and GLEY not only does an exterior activation of the trypsinogen in the juice take place, but also in the interior of the gland. An auto-activation of the juice in certain cases is also accepted by others (SAWITSCH [2]).

The activation of the trypsinogen into trypsin may, in life, be brought about —as the researches of HERZEN, which have been substantiated by GACHET and PACHON, BELLAMY, MENDEL and RETTGER, have shown—not only in the intestine, but also in the gland itself. This activation of the trypsinogen in the gland itself is caused in a still undiscovered manner by a body of unknown nature formed in the spleen, which is congested during digestion. Such a " charging " of the pancreas by the spleen has been repeatedly suggested by SCHIFF,[3] but this has recently been denied by PRYM. According to this experimenter the extirpation of the spleen causes no change in the properties of the pancreatic juice, and the intravenous injection of spleen infusion is also without action on a splenectomized dog with permanent pancreatic fistula. The observations of HERZEN that a spleen infusion has a strong activating action upon a weak pancreas infusion were substantiated by PRYM,[4] but he claims that this is due essentially to micro-organisms.

The formation of lactase after the introduction of milk sugar into the intestine as observed by WEINLAND and BAINBRIDGE, is to be considered as an intraglandular enzyme formation in the pancreas. This is a special example of the general rule based upon BROCARD'S researches, that the kind of food has a marked influence upon the formation of hydrolytic ferments in the body: " c'est l'ailment qui fait le ferment." This formation of lactase is denied by others such as BIERRY and PLIMMER.[5]

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[1] Compt. rend. soc. biol., **63**.

[2] Camus and Gley, Journ. de Physiol. et de Pathol. gén., 1907; Zunz, Recherches sur l'activation de sac pancreatique par les Sels., Bruxelles, 1907; Sawitsch, Zentralbl. f. d. ges. Physiol. u. Path. des Stoffwechsels, 1909.

[3] Bellamy, Journ. of Physiol., **27**; Mendel and Rettger, Amer. Journ. of Physiol., **7**. A very complete reference to the literature may be found in Menia Besbokaia Du rapport fonctionell entre le pankréas et la rate, Lausanne, 1901.

[4] Pflüger's Arch., **104** and **107**.

[5] Weinland, Zeitschr. f. Biologie, **38** and **40**; Brocard, Journ. de physiol. et de path. gén., **4**; Bainbridge, Journ. of Physiol., **31**. Contradictory views are held

The conversion of the trypsinogen into trypsin in the removed gland or in an infusion under the influence of air and water and also by other bodies has been known for a long time. According to VERNON the trypsin itself has a strong activating action upon trypsinogen, and in this regard it is more active than enterokinase. The correctness of this statement is still denied by BAYLISS and STARLING and by HEKMA. The ordinary view of HEIDENHAIN, that the transformation of trypsinogen into trypsin is also brought about by acids, has been found to be incorrect by HEKMA.[1] Besides the enterokinase and the micro-organisms, there are other activators of the trypsinogen, namely liver-press-juice (WOHLGEMUTH [2]) and certain amino-acids and finally, as first shown by DELEZENNE and then by ZUNZ by further investigations, especially the lime salts.[3] These last do not act immediately, but only after some time, for example, a couple of hours, and then they activate suddenly. The lime salts are not necessary for the digestive action of the juice, and when the activation has once taken place, they can be removed without any harm. They probably have a similar action as in the coagulation of the blood. According to DELEZENNE the lime salts have the same importance in the activation of the rennin-zymogen of the juice as in the activation of the trypsinogen. This enzyme is also activated by enterokinase.

The way in which the trypsinogen is converted into trypsin is still unknown and is the subject of dispute. According to one view, proposed by PAWLOW and defended by BAYLISS and STARLING, the trypsinogen is transformed into trypsin by the action of the kinase. In the opinion of DELEZENNE, DASTRE, and STASSANO, and others,[4] the trypsin, on the contrary, is a combination of the kinase and trypsinogen, analogous to the hæmolysins, which according to EHRLICH'S side-chain theory are combinations between a complement and an amboceptor.

The specific excitants for the secretion of pancreatic juice are, according to PAWLOW and his collaborators, acids of various kinds—hydrochloric acid as well as lactic acid—and fats, the latter acting probably by virtue of the soaps produced therefrom. Alkalies and alkali carbonates have, on the contrary, a retarding action. It appears that the acids act by irritating the mucosa of the duodenum. Water, which causes a secretion of acid gastric juice, likewise becomes, indirectly, a stimulant for the

by Bierry, Compt. rend., **140**, and Compt. rend. soc. biolog., **58**, and Plimmer, Journ. of Physiol., **34**.

[1] Vernon, Journ. of Physiol., **28**; Hekma, Kon. Akad. v. Wetenschappen te Amsterdam, 1903, and Arch. f. (Anat. u.) Physiol., 1904; Bayliss and Starling, Journ. of Physiol., **30**.

[2] Bioch. Zeitschr., **2**.

[3] Delezenne, Compt. rend. soc. biol., **59**, **60**, **62**, **63**; Zunz, footnote 2, p. 473.

[4] Bayliss and Starling, Journ. of Physiol., **30** and **32**, which also cites the other investigators. See also footnote 1, p. 472.

pancreatic secretion, but may also be an independent exciter. The psychical moment may, at least in the first place, have an indirect action (secretion of acid gastric juice), and the food seems otherwise to have an action on pancreatic secretion by its action on the secretion of gastric juice.

The most important excitant for the secretion of juice is hydrochloric acid, but opinions are not in unison as to the manner in which the acid acts. PAWLOW'S school claims that the acid acts reflexly upon the intestine, causing a secretion of juice. That a reflex action is here produced is not contradicted by the investigations of POPIELSKI, WERTHEIMER and LEPAGE, FLEIG,[1] and others. According to the researches of BAYLISS and STARLING, which have been confirmed by CAMUS, GLEY, FLEIG, HERZEN, DELEZENNE, and others, a second factor must also be active here. BAYLISS and STARLING have shown that a body which they have called *secretin* can be extracted from the intestinal mucosa by a hydrochloric-acid solution of 4 p. m., and this when introduced into the blood produces a secretion of pancreatic juice, bile, and in the opinion of some investigators also of saliva and intestinal juice. The secretin, which according to BAYLISS and STARLING [2] is the same in all vertebrates examined, is not destroyed by heat; it is therefore not identical with enterokinase, and is not considered an enzyme. It is formed from another substance, *prosecretin*, by the action of acids. According to DELEZENNE and POZERSKI [3] secretin occurs as such in the intestinal mucosa, and the acids act only by the destruction of certain bodies having a retarding action. According to POPIELSKI secretin action is different from acid action: and the secretin action can also be obtained by WITTE'S peptone. He believes that the secretin is not a specific constituent of the intestine but a body widely distributed. On the introduction of intestinal extract into the animal body the blood-pressure is suddenly and very strongly reduced, the blood becomes uncoagulable and a secretion of saliva, tears, pancreatic juice, gastric juice and bile (not constantly) occurs. The same action is also produced by the injection of an aqueous extract of other organs, and the action is not due to choline, but to a special substance, which is called *vasodilatin*, and which also occurs in WITTE'S peptone. The proteoses and peptones are themselves without action and the view of PICK and SPIRO (page 311), that their action upon the coagulation of the blood is due to a contaminating substance, a peptozyme, seems to be correct. POPIELSKI and PANEK have suggested another method of

[1] Fleig, Centralbl. f. Physiol., **16**, 681, and Compt. rend. soc. biol., **55**. See also foot-note 1, p. 472.

[2] Journ. of Physiol., **29**.

[3] Delezenne and Pozerski, Compt. rend. soc. biol., **56**; Popielski, Centralbl. f. Physiol., **19**.

preparing vasodilatin.[1] GIZELT disputes the occurrence of a specific secretin and considers this body a peptone. v. FÜRTH and SCHWARZ[2] also call attention to the uncertainty of our knowledge as to the nature of secretin. According to them secretin is probably a mixture of bodies, among which probably the choline, found by them in the intestinal walls, acts the role of a secretin exciter.

A second means of causing secretion is the fat, which probably only acts after it has been saponified. Oil-soap directly introduced into the duodenum brings about a strong secretion of pancreatic juice (SAWITSCH, BABKINE[3]), and at the same time a flow of bile, gastric juice, and the secretion of BRUNNER'S glands occurs. The pancreatic juice secreted under these circumstances has about the same amount of enzymes as the juice secreted after partaking of food. We know very little as to how the soaps act. FLEIG[4] found that by maceration of the mucosa of the upper part of the duodenum with soap solution a substance goes into solution which he calls *sapokrinin* and which when introduced into the blood brings about a strong secretion of pancreatic juice. This sapokrinin, which is derived from a prosapokrinin, is not an enzyme and is not identical with secretin. After the action of chloral hydrate an abundant secretion occurs in the duodenum (WERTHEIMER and LEPAGE), which FALLOISE considers as produced by a special secretin, chloral secretin. The secretion of pancreatic juice can also be increased by alcohol and FLEIG[5] claims to have obtained a secretin, *ethyl secretin* by macerating the intestinal mucosa with alcohol. Further investigations are necessary of all these so-called secretins.

The estimation as to the quantity of pancreatic juice secreted in the twenty-four hours differs very much. According to the determinations of PAWLOW and his collaborators, KUWSCHINSKI, WASSILIEW, and JABLONSKY,[6] the average quantity (with normally acting juice) from a permanent fistula in dogs is 21.8 cc. per kilo in the twenty-four hours. GLAESSNER[7] found in man in one case 600–800 grams in the 24 hours.

The pancreatic juice of the dog is a clear, colorless, and odorless alkaline fluid which when obtained from a temporary fistula is very

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[1] Popielski, Pflüger's Arch., **128**; Popielski and Panek, *ibid.*, **128**.

[2] Gizelt, Pflüger's Arch., **123**; v. Fürth and Schwarz, *ibid.*, **124** (literature on secretin).

[3] Arch des scienc. biol. de St. Pétersbourg, **11**, and Zeitschr. f. physiol. Chem., **56**.

[4] Compt. rend. soc. biol., **55**, and Journ. de Physiol. et de Pathol. gén., 1904.

[5] Wertheimer and Lepage, Compt. rend. soc. biol., **52**; Fleig, *ibid.*, **55**; Falloise, Bull. Acad. Roy. Belg., 1903.

[6] Arch. des sciences de St. Pétersbourg, **2**, 391. The previous claims of Keferstein and Hallwachs, Bidder and Schmidt, and others may be found in Kühne, Lehrbuch, 114.

[7] Zeitschr. f. physiol. Chem., **40**.

rich in proteins, sometimes so rich that it coagulates like the white of the egg on heating. Besides *proteins*, the juice also contains the three above-mentioned enzymes (or their zymogens), *amylopsin*, perhaps also *maltase*, *trypsin*, *steapsin*, also an enzyme similar to erepsin, and besides these a *rennin*, which was first observed by KÜHNE. Besides the above-mentioned bodies the pancreatic juice invariably contains small quantities of *leucine*, *fat*, and *soaps*. As mineral constituents it contains chiefly alkali chlorides and considerable alkali carbonate, some phosphoric acid, lime, magnesia, and iron.

The quantity of solids in the pancreatic juice of the dog varies, as found by MAZURKIEWICZ, BABKINE and SAWITSCH,[1] according to the rapidity of secretion and the kind of excitant. As a rule the amount of solids is in inverse proportion to the rapidity of secretion. The juice secreted after the action of acids has the lowest amount of solids, 9–37.4 p. m. The juice after taking food is more concentrated, about 60–70 p. m. and that after vagus stimulation often contains 90 p. m. solids. The juice analyzed by C. SCHMIDT[2] from a temporary fistula contained 99–116 p. m. solids. The quantity of mineral bodies was 8.8 p. m.

The mineral constituents consisted chiefly of NaCl, 7.4 p. m., which is remarkable because the juice contains such a large amount of alkali carbonate. In the juice examined by DE ZILWA[3] the quantity of alkali in the secretin juice was 5–7.9 p. m. and in the pilocarpin juice 2.9–5.3 p. m. Na_2CO_3.

In the pancreatic juice of rabbits 11–26 p. m. solids have been found, and in that from sheep 14.3–36.9 p. m. In the pancreatic juice of the horse 9–15.5 p. m. solids have been found; in that of the pigeon, 12–14 p. m.

The human physiological pancreatic secretion from a fistula has been investigated by GLAESSNER.[4] The secretion was clear, foamed readily, had a strong alkaline reaction even toward phenolphthalein, and contained globulin and albumin but no proteoses and peptones. The specific gravity was 1.0075 and the freezing-point depression was $\Delta = -0.46$–$0.49°$. The solids were 12.44–12.71 p. m., the total protein 1.28–1.74 p. m., and the mineral bodies 5.66–6.98 p. m. The secretion contained trypsinogen, which was activated by the intestinal juice. Diastase and lipase were present; inverting enzymes, on the contrary, were not. The daily quantity of juice was 500–800 cc. The quantity of secretion, of ferments, and of alkalinity was lowest in starvation, but soon rose with the taking of food, and reached its maximum in about four hours.

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[1] Mazurkiewicz, l. c.; Babkine and Sawitsch, Zeitschr. f. physiol Chem., 56.

[2] Cited from Maly in Hermann's Handbuch der Physiol., 5, Theil II, 189.

[3] Journ. of Physiol., 31.

[4] Zeitschr. f. physiol. Chem., 40. See also Ellinger and Kohn, *ibid.*, 45, and the investigations upon cystic fluids from the pancreas by Schumm, *ibid.*, 36, and Murray and Gies, American Medicine, 4, 1902.

Amylopsin or **pancreatic diastase,** which, according to KOROWIN and ZWEIFEL, is not found in new-born infants and does not appear until more than one month after birth, seems, although not identical with ptyalin, to be closely related to it. Amylopsin acts very energetically upon boiled starch, and according to KÜHNE also upon unboiled starch, especially at 37° to 40° C., and according to VERNON [1] best at 35° C. It forms, similarly to the action of saliva, besides dextrin, chiefly isomaltose and maltose, with only very little dextrose (MUSCULUS and v. MERING, KÜLZ and VOGEL [2]). The dextrose is probably formed by the action of the invertin existing in the gland and juice. The pancreatic juice of the dog in fact, contains, according to BIERRY and TERROINE,[3] maltase, its action becomes apparent only after very faint acidification of the juice. According to RACHFORD the action of the amylopsin is not reduced by very small quantities of hydrochloric acid, but is diminished by larger amounts. VERNON, GRÜTZNER, and WACHSMANN [4] find that the action is indeed accelerated by very small quantities of hydrochloric acid, 0.045 p. m. while alkalies in very small amounts have a retarding action. This retarding action of alkalies and hydrochloric acid may be stopped by bile (RACHFORD.)

Steapsin or **Fat-splitting Enzyme.** The action of the pancreatic juice on fats is twofold. First, the neutral fats are split into fatty acids and glycerin, which is an enzymotic process; and secondly, it has also the property of emulsifying the fats.

The action of the pancreatic juice in splitting the fats may be shown in the following way: Shake olive-oil with caustic soda and ether, siphon off the ether and filter if necessary, then shake the ether repeatedly with water and evaporate at a gentle heat. In this way is obtained a residue of fat free from fatty acids, which is neutral and which dissolves in acid-free alcohol and is not colored red by alkanet tincture. If such fat is mixed with perfectly fresh alkaline pancreatic juice or with a freshly prepared infusion of the fresh gland and treated with a little alkali or with a faintly alkaline glycerin extract of the fresh gland (9 parts glycerin and 1 part 1 per cent soda solution for each gram of the gland), and some litmus tincture added and the mixture warmed to 37° C., the alkaline reaction will gradually disappear and an acid one take its place. This acid reaction depends upon the conversion of the neutral fats by the enzyme into glycerin and free fatty acids. A very much used method consists in determining the acidity of the mixture by means of titration before and after the action of the juice or the infusion.

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[1] Korowin, Maly's Jahresber., **3**; Zweifel, footnote 3, p. 431; Kühne, Lehrbuch, 117; Vernon, Journ. of Physiol., **27**.

[2] See footnote 1, p. 432.

[3] See Tebb, Journ. of Physiol., **15**; Bierry and Terroine, Compt. rend. soc. biolog., 58; Bierry, *ibid.*, 62.

[4] Rachford, Amer. Journ. of Physiol., **2**; Vernon, l. c.; Grützner, Pflüger's Arch., **91**.

The action of the pancreatic juice in splitting fats is a process analogous to that of saponification, the neutral fats being decomposed, by the addition of the elements of water into fatty acids and glycerin according to the following equation. $C_3H_5.O_3.R_3$ (neutral fat) $+3H_2O=$ $C_3H_5.O_3.H_3$ (glycerin) $+3$(H.O.R) (fatty acid). This depends upon a hydrolytic splitting, which was first positively proven by BERNARD and BERTHELOT. The pancreas enzyme also decomposes other esters, just as it does the neutral fats (NENCKI, BAAS, LOEVENHART [1] and others). The fat-splitting enzyme of the pancreas is, according to PAWLOW and BRUNO and many others [2] aided in its action by the bile, and according to ENGEL obeys SCHÜTZ-BORISSOW'S rule that the extent of cleavage during a given time is proportional to the square root of the quantity of ferment. The investigations of KANITZ [3] have led to the same results.

POTTEVIN [4] found that the pancreas (free from water) could form olein from oleic acid and glycerin. It is claimed that the gland can form other esters from oleic acid or stearic acid with other alcohols (amyl alcohol) if we operate only in the absence of water. In the presence of considerable water the pancreas has a reverse saponifying action. (See page 65).

The fatty acids which are split off by the action of the pancreatic juice combine in the intestine with the alkalies, forming soaps, which have a strong emulsifying action on the fats, and thus the pancreatic juice becomes of great importance in the emulsification and the absorption of the fats.

Trypsin. The action of the pancreatic juice in digesting proteins was first observed by BERNARD, but first proven by CORVISART.[5] It depends upon a special enzyme called by KÜHNE trypsin. This enzyme, as previously explained, does not occur in the gland as such, but as trypsinogen. According to ALBERTONI [6] this zymogen is found in the gland in the last third of the intra-uterine life. Enzymes more or less like trypsin occur in other organs, and are very widely diffused in the vegetable

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[1] Bernard, Ann. de chim. et physique (3), **25**; Berthelot, Jahresber. d. Chem., 1855, 733; Nencki, Arch. f. exp. Path. u. Pharm., **20**; Baas, Zeitschr. f. physiol. Chem., **14**, 416; Loevenhart, Journ. of Biol. Chem., **2**; Terroine and Z. Morel, Compt. rend. soc. biol., **65**, **66**.

[2] Bruno, Arch. des scienc. biol. de St. Pétersbourg, **7**; see also Loevenhart and Souder, Journ. of biol. Chem., **2**; v. Fürth and Schütz, Hofmeister's Beiträge, **9**; Kalaboukoff and Terroine, Compt. rend. soc. biol., **63**.

[3] Engel, Hofmeister's Beiträge, **7**; Kanitz, Zeitschr. f. physiol. Chem., **46**.

[4] Compt. rend., **138**; Annal. Institute Pasteur, **20**, and Bull. soc. Chem. (3), **35**.

[5] Gaz. hebdomadaire, 1857, Nos. 15, 16, 19, cited from Bunge, Lehrbuch, 4. Aufl., 185.

[6] See Maly's Jahresber., 8, 254.

kingdom,[1] in yeast and in higher plants, and are also formed by various bacteria. The enzymes similar to trypsin occurring in the plant kingdom are, according to VINES, a mixture of *peptases*, which transform the proteins into peptone and *ereptases*, which split the peptones into amino-acids.

As we know of so-called antienzymes for other enzymes, so we also have antitrypsins, and not only in the intestinal canal but also in the blood-serum. The results as to the specificity of these antitrypsins in various animals, as well as the possibility of producing antitrypsins by immunization, is still disputed.

Trypsin, like other enzymes, has not been prepared in a pure condition. Nothing is positively known in regard to its nature, but as obtained thus far it shows a variable behavior (KÜHNE, KLUG, LEVENE, MAYS, and others). At least it does not seem to be a nucleoprotein, and trypsin has also been obtained which did not give the biuret test (KLUG, MAYS, SCHWARZCHILD). Trypsin dissolves in water and glycerin, while KÜHNE'S trypsin is insoluble in glycerin. It is very sensitive to heat, and even the body temperature gradually decomposes it (VERNON, MAYS). In neutral solution it becomes inactive at 45° C. In dilute soda solution of 3–5 p. m. it is still more readily destroyed (BIERNACKI, VERNON [2]). The presence of protein or proteoses has, to a certain extent, a protective action on heating an alkaline trypsin solution, and this has been substantiated by recent investigations of BAYLISS and VERNON. The simpler cleavage products have a still greater protective action (VERNON [3]). Trypsinogen, according to the unanimous statements of several experimenters, is more resistant toward alkalies than trypsin. Trypsin is gradually destroyed by gastric juice and even by digestive hydrochloric acid alone.

The preparation of pure trypsin has been tried by various experimenters. The most careful work in this direction was done by KÜHNE and MAYS. Various methods have been suggested by MAYS, but we cannot enter into a discussion of them. A very pure preparation can be obtained by making use of the combined salting out with NaCl and $MgSO_4$. A very active solution, and one that can be kept for a long time (for more than twenty years according to HAMMARSTEN), can be obtained by extracting with glycerin (HEIDENHAIN [4]). An impure but still very active infusion

[1] In this connection see Vines, Annals of Botany, **16**, **17**, **18**, **19**, **22**, and **23**, and Oppenheimer, Die Fermente, 1900.

[2] Kühne, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**, 3; Klug, Math. naturw. Ber. aus Ungarn., **18**, 1902; Levene, Amer. Journ. of Physiol., **5**; Mays, Zeitschr. f. Physiol. Chem., **38**; Vernon, Journ. of Physiol., **28** and **29**; Biernacki, Zeitschr. f. Biologie, **28**; Schwarzschild, Hofmeister's Beiträge, **4**.

[3] Bayliss, Arch. des scienc. biolog. de St. Pétersbourg, **11**, Suppl.; Vernon, Journ. of Physiol., **31**.

[4] Pflüger's Arch., **10**.

can be obtained after a few days by allowing the finely divided gland to stand with water which contains 5–10 cc. chloroform per liter (SALKOWSKI) at the temperature of the room. This infusion can be kept very active for several years at the cellar temperature. For digestion experiments the active commercial trypsin preparations can be employed.

Like other enzymes, trypsin is characterized by its action, and this action consists in dissolving protein and in splitting it into simpler products, mono- and diamino-acids, tryptophane, etc., in alkaline, neutral, and indeed in very faintly acid solutions. This action has been so far considered as characteristic for trypsin. Recent investigations seem to indicate that this action is not due to one enzyme alone, but to the combined action of several enzymes.

Although contrary to MAY'S statement, there is no question that there occurs in the pancreas besides trypsin an enzyme similar to erepsin (BAYLISS and STARLING, VERNON [1]). According to the latter this erepsin has a strong action upon peptone, and he believes that the peptone-splitting action of a pancreas infusion is in great part due to the erepsin. The pancreas besides these also contains a *nuclease* (see page 469), whose relation to pancreas erepsin has not been determined.

The unity of trypsin has also been disputed from another point of view. According to POLLAK the trypsin (in the ordinary sense) contains a second enzyme, which does not act upon protein, but only upon gelatin, and he calls this enzyme *glutinase*. This glutinase is much more resistant toward acids than trypsin, and by proper treatment with acids POLLAK was able to change a pancreas infusion so that it acted upon gelatin and not upon certain proteins. The correctness of these observations has, indeed, not been generally accepted, and it is disputed by ASCOLI and NEPPI.[2] According to them the action of the trypsin is weakened by the acid, and indeed to such varying degrees for different proteins that the action upon albumin is lost while the action upon gelatin is noticeable. Nevertheless, we here have a warning to be careful as to the conclusions drawn from results where impure infusions are used. For many experiments it is undoubtedly advisable to use the natural pancreatic juice.

The following reports on the action of trypsin applies to the so-called trypsin, with the reservation that it is perhaps not a unit enzyme.

The *action of trypsin on proteins* is best demonstrated by the use of fibrin. Very considerable quantities of this protein body are dissolved by a small amount of trypsin at 37–40° C. It is always necessary to make a control test with fibrin alone, with or without the addition of alkali. Fibrin is dissolved by trypsin without any putrefaction; the liquid has a pleasant odor somewhat like bouillon. To completely exclude putrefaction a little thymol, chloroform, or toluene should be added to the

[1] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30**; and Zeitschr. f. physiol. Chem., **50**; Mays, *ibid.*, **49** and **51**.

[2] Pollak, Hofmeister's Beiträge, **6**; contradictory statements are found in Ehrenreich, cited in Bioch. Centralbl., **4**; Ascoli and Neppi, Zeitschr. f. physiol. Chem., **56**.

liquid. Tryptic digestion differs essentially from pepsin digestion, irrespective of the difference in the digestive products, in that the first takes place in neutral or alkaline reaction and not, as is necessary for peptic digestion, in an acidity of 1–2 p. m. HCl, and further by the fact that the proteins dissolve in trypsin digestion without previously swelling up.

As trypsin not only dissolves proteids, but also other protein substances such as gelatin, this latter body may be used in detecting trypsin. The liquefaction of strongly disinfected gelatin is, according to FERMI,[1] a very delicate test for trypsin or tryptic enzymes. Various suggestions for the use of gelatin in the trypsin test have been made. In consideration of the observations of ASCOLI and NEPPI that a trypsin may not act upon fibrin or other proteids but still digest gelatin, it is advisable never to make use of gelatin or proteid alone in testing for trypsin, but always the two.

For the quantitative estimation of trypsin by measuring the rapidity of digestion we generally make use of the method of METT, described under pepsin digestion. Another method, suggested by WEISS, consists in determining the nitrogen in the filtrate after coagulation with heat and acetic acid. LÖHLEIN recommends the titration method of VOLHARD as used in pepsin determinations, and has given directions for its use. JACOBY recommends the use of ricin and GROSS suggests a method based upon the precipitation of casein by acid. BAYLISS follows the digestion by the electrical conductivity, and HEDIN [2] determines the quantity of nitrogen not precipitated by tannic acid.

Many circumstances exert a marked influence on the *rapidity of the trypsin digestion.* With an increase in the *quantity of enzyme* present the digestion is hastened, at least to a certain point. According to PAWLOW and his school, the rule of SCHÜTZ-BORISSOW is perfectly applicable to trypsin, and the amount digested is proportional to the square root of the quantity of ferment. This does not agree with the results of BAYLISS, HEDIN and others, making use of other methods of determination. HEDIN,[3] who has especially studied this question, found under certain conditions a direct proportion between the quantity of enzyme and the intensity of digestion as explained in detail on pages 62, 63. Tryptic digestion is also accelerated by an increase of *temperature,* at least to about 40° C., at which temperature the protein is very rapidly dissolved by the trypsin. The *reaction* is also of the greatest importance. Trypsin acts energetically in neutral, or still better in alkaline, solutions, and according to older statements, best in an alkalinity of 3–4 p. m. Na_2CO_3;

[1] Arch. f. Hyg., **12** and **55**.

[2] Weiss, Zeitschr. f. physiol. Chem., **40**; Löhlein, Hofmeister's Beiträge, **7**; Jacoby, Bioch. Zeitschr., **10**; Gross, Arch. f. exp. Path. u. Pharm., **58**; Bayliss, Arch. des scienc. biol. de St. Pétersbourg, **11**, Suppl.; and Journ. of Physiol., **36**; Hedin, *ibid.*, **32**.

[3] Pawlow, Die Arbeit der Verdauungsdrüsen, Wiesbaden, 1898, p. 33; Bayliss, l. c.; Hedin, l. c.; and Chapter II, pp. 62 and 63.

but the nature of the protein is also of importance. The reports in regard to the action of trypsin in various reactions are still somewhat disputed.[1] The action of the alkali depends upon the number of hydroxyl ions (DIETZE, KANITZ), and according to KANITZ [2] the digestion proceeds best in those solutions which are 1/70–1/200 normal in regard to hydroxyl ions. Free mineral acids, even in very small quantities, completely prevent the digestion. If the acid is not actually free, but combined with protein bodies, then the digestion may take place quickly when the acid combination is not in too great excess (CHITTENDEN and CUMMINS). Organic acids act less disturbingly, and in the presence of 0.2 p. m. lactic acid and the simultaneous presence of bile and common salt the digestion may indeed proceed more quickly than in a faintly alkaline liquid (LINDBERGER). The assertion of RACHFORD and SOUTHGATE, that the bile can prevent the injurious action of the hydrochloric acid, and that a mixture of pancreatic juice, bile, and hydrochloric acid digests better than a neutral pancreatic juice, could not be substantiated by CHITTENDEN and ALBRO. That bile has an action tending to aid the tryptic digestion has been shown by many investigators, and recently by BRUNO, ZUNTZ and Ussow and others.[3]

Carbon dioxide, according to SCHIERBECK,[4] has a retarding action in acid solutions, but it accelerates the tryptic digestion in faintly alkaline liquids. *Foreign bodies*, such as potassium cyanide, may promote tryptic digestion, while other bodies, such as salts of mercury, iron, and others (CHITTENDEN and CUMMINS), or salicylic acid in large quantities, may have a preventive action. According to WEISS [5] the halogen alkali salts disturb tryptic digestion only slightly, and NaCl seems to have the strongest action. The sulphates have a much stronger retarding action than the chlorides. The *nature of the proteins* is also of importance. Unboiled fibrin is, relatively to most other proteins, dissolved so very quickly that the digestion test with raw fibrin gives an incorrect idea of the power of trypsin to dissolve coagulated protein bodies in general. Boiled fibrin is digested with much greater difficulty and also requires a higher alkalinity: 8 p. m. Na_2CO_3 (VERNON [6]). The resistance of certain native protein solutions, such as blood-serum and egg-

[1] See Kudo, Bioch. Zeitschr., **15**.

[2] Kanitz, Zeitschr. f. physiol. Chem., **37**, who also cites Dietze.

[3] Chittenden and Cummins, Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 1885, **1**, 100; Lindberger, Maly's Jahresber., **13**; Rachford and Southgate, Medical Record, 1895; Chittenden and Albro, Amer. Journ. of Physiol., **1**, 1898; Rachford, Journ. of Physiol., **25**; Bruno, l. c.; Zuntz and Ussow, Arch. f. (Anat. u.) Physiol., 1900.

[4] Skand. Arch. f. Physiol., **3**.

[5] Weiss, Zeitschr. f. physiol. Chem., **40**. See also Kudo, l. c.

[6] Journ. of Physiol., **28**.

white, against the action of trypsin is remarkable—a behavior which can be explained by the occurrence of anti-bodies in these solutions and which HEDIN [1] has carefully studied. An *accumulation of the products of indigestion* tends to hinder the trypsin digestion.

An interesting contrast to the inactivity of trypsin toward native egg-albumin and seralbumin is the enzyme *papain*, which, as the investigations of DELEZENNE, MOUTON and POZERSKI and of JONESCU and SACHS [2] have shown, is hindered in its action by an anti-body in the albumin which is destroyed by heat or by the action of acid.

The Products of the Tryptic Digestion. In the digestion of unboiled fibrin a globulin which coagulates at 55–60° C. may be obtained as an intermediate product (HERRMANN [3]). Besides this one obtains from fibrin, as well as from other proteins, the products previously mentioned in Chapter III. In trypsin digestion the cleavage may proceed so far that the mixture fails to give the biuret reaction. This does not indicate, as E. FISCHER and ABDERHALDEN have shown, a complete cleavage of the protein molecule into mono- and diamino-acids, etc. In tryptic digestion, as shown by ABDERHALDEN and REINBOLD, using the protein edestin, and by ABDERHALDEN and VOEGTLIN,[4] with casein, a gradual cleavage of the protein takes place, and thereby certain amino-acids, like tyrosine and tryptophane, are readily and completely split off, while others, like leucine, alanine, aspartic acid, and glutamic acid, are slowly and less readily split off, and others, such as α-proline, phenylalanine, and glycocoll, stubbornly resist the cleavage action of the trypsin. The polypeptide-like bodies discovered by FISCHER and ABDERHALDEN, which are produced in digestion, and which do not give the biuret reaction, are the atomic complexes which resist the action of trypsin. These peptoids contain the pyrrolidine carboxylic acid and phenylalanine groups of the protein, but also yield other monamino-acids such as leucine, alanine, glutamic acid, and aspartic acid. Among the above-mentioned products we find on the autodigestion of the gland other substances, such as oxyphenylethylamine (EMERSON), which is produced from tyrosine by fermentive CO_2 cleavage, also uracil (LEVENE), guanidine (KUTSCHER and OTORI), the purine bases, which originate from the nuclein bodies, and choline, which latter is formed from lecithin (KUTSCHER and LOHMANN [5]). If putrefaction is not completely prevented,

[1] See Zeitschr. f. physiol. Chem., **50**, where the works of Hedin are cited.

[2] Delezenne, Mouton and Pozerski, Compt. rend., **142**; Jonescu, Bioch. Zeitschr., **2**, Sachs, Zeitschr. f. physiol. Chem., **51**.

[3] Herrmann, Zeitschr. f. physiol. Chem., **11**.

[4] Abderhalden and Reinbold, Zeitschr. f. physiol. Chem., **44** and **46**, with Voegtlin, *ibid.* **53**.

[5] Fischer and Abderhalden, Zeitschr. f. physiol. Chem., **39**; Emerson, Hofmeister's

still other bodies occur which will be considered later in connection with the putrefactive processes in the intestine.

The Action of Trypsin upon other Bodies. The *neucleoproteins* and *nucleins* are so digested that the protein complex is separated from the nucleic acid and then digested. The nucleic acids may, nevertheless, be somewhat changed (Araki), which is probably brought about by another enzyme, the *nuclease* (Sachs). A cleavage of nucleic acids with the setting free of phosphoric acid and purine bases is, according to Iwanoff,[1] not brought about by trypsin. The splitting is first produced by the action of nuclease or erepsin (see page 467). *Gelatin* is dissolved and digested by pancreatic juice. A cleavage with the separation of glycocoll and leucine does not occur (Kühne and Ewald), or only to a trivial extent (Reich-Herzberge [2]).

The *gelatin-forming substance* of the connective tissues is not directly dissolved by trypsin, but only after it has been treated with acids or soaked in water at 70° C. By the action of trypsin on hyaline *cartilage* the cells dissolve, leaving the nucleus. The matrix is softened and shows an indistinctly constructed network of collagenous substance (Kühne and Ewald). The *elastic substance,* the *structureless membranes,* and the *membrane of the fat-cells,* are also dissolved. *Parenchymatous organs,* such as the liver and the muscles, are dissolved all but the nuclei, connective tissue, fat-corpuscles, and the remainder of the nervous tissue. If the muscles are boiled, then the connective tissue is also dissolved. *Mucin* is dissolved and split by trypsin, while *chitin* and *horn substance* do not seem to be acted upon by the enzyme. *Oxyhæmoglobin* is decomposed by trypsin with the splitting off of hæmatin. Trypsin has no action upon fats and carbohydrates.

The action of trypsin on simply constructed substances of known constitution such as acid-amides, polypeptides, is of especially great interest. In this regard we have the somewhat earlier investigations of Gulewitsch, Gonnermann, and Schwarzschild,[3] but the investigations of Fischer and of Abderhalden and his co-workers,[4] are much more complete and important.

Beiträge, **1**; Levene, Zeitschr. f. physiol. Chem., **37**; Kutscher and Lohmann, *ibid.*, **39**; Kutscher and Otori, *ibid.*, **43**, and Centralbl. f. Physiol., **18**.

[1] Iwanoff, Zeitschr. f. physiol. Chem., **39**, which also contains the literature; Sachs, *ibid.*, **46**.

[2] Kühne and Ewald, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**; Reich-Herzberge, Zeitschr. f. physiol. Chem., **34**.

[3] Hofmeister's Beiträge, **4**, where the other works are also cited.

[4] Fischer and Bergell, Ber. d. d. chem. Gesellsch., **36** and **37**; Fischer and Abderhalden, Sitzungsber. der Kgl. Pr. Akad. d. Wissensch., Berlin, 1905. The works of Abderhalden and co-workers cannot be specially cited, but may be found in Zeitschr. f. physiol. Chem., **47**, **48**, **49**, **51**, **52**, **53**, **54**, **55**, and **57**.

As has been indicated (Chapter III) only those peptides are split hydrolytically by enzymes which are formed from the amino-acids occurring in nature. It has also been shown that with this limited action of trypsin or the pancreatic juice only a certain number of peptides, di-, tri-, as well as tetrapeptides are split, while it is without action upon a number of others. The structure of these plays an important rôle, as, for example, alanyl-glycine, $CH_3.CH(NH_2).CO.NH.CH_2.COOH$, is split, while its isomer glycl-alanine, $NH_2.CH_2.CO.NH.CH(CH_3).COOH$, is, on the contrary, not split. The nature of the amino-acids existing in the peptide is also of importance. Those dipeptides which contain alanine as acyl—for example, alanylglycine, alanyl-alanine, and alanyl-leucine A—are readily hydrolyzed, while several dipeptides in which α-aminobutyric acid or leucine functionates as acyl are very resistant. The number of amino-acid groups is also of importance, as, for example, triglycyl-glycine is not split, while tetraglycyl-glycine is. In those peptides which are racemic bodies the hydrolysis takes place asymmetrically, so that (see Chapter III) only one-half of the racemic body is attacked, and those active amino-acids result as products which are contained in the natural protein bodies.

The behavior of the polypeptides with trypsin, or nearly related enzymes, is of the very greatest interest and in many regards very important. Thus in the polypeptides we have a means of determining the kind of enzyme, whether it belongs to the pepsin, trypsin, or erepsin group. We know of no polypeptide which is split by pepsin; trypsin splits only certain polypeptides, but not others, while the erepsin on the contrary seems to split all polypeptides, which are composed of amino-acids, occurring in nature. By the aid of the polypeptide reaction ABDERHALDEN and co-workers have also been able to show that the trypsin-like enzyme, occurring in the blood plasma, is not identical with trypsin because it does not attack glycyl-*l*-tyrosine, which is split by trypsin.

By observations upon the optical behavior of a solution of a certain optically active polypeptide on the addition of enzyme we can follow the decomposition step by step, as many polypeptides have a stronger rotation than their cleavage products, or as a change in the direction of the rotation occurs during decomposition. In this manner we can determine the enzyme hydrolysis in a certain time and in this way the interesting investigations of ABDERHALDEN and GIGON [1] have shown that the hydrolysis of glycyl-*l*-tyrosine with yeast press-fluid is retarded by the addition of optically active amino-acids occurring in nature, while the optical antipodes of the same amino-acids is without action or at least have only a very weak retarding action. An analogous condition in digestion

[1] Zeitschr. f. physiol., Chem. 53.

explains in part why the decomposition of proteins *in vitro* is slower than in the digestive tract, where the decomposition products are removed by absorption. On following the changes in the optical behavior during the enzymotic cleavage of polypeptides one can, as ABDERHALDEN with KOELKER and BRAHM [1] have shown, determine at which point the enzyme first applies its action in the cleavage of a tripeptide. We cannot enter into further detail as regard this and the other related behaviors.

Pancreatic rennin is an enzyme found in the gland and in the juice, which coagulates neutral or alkaline milk (KÜHNE and ROBERTS and others). This enzyme, according to PAWLOW'S school, is identical with trypsin. The similarity of action of these two enzymes and the fact that both are activated simultaneously from the zymogens by enterokinase or lime salts (DELEZENNE, WOHLGEMUTH [2]) seem to point to this identity. On the other hand the optimum of the enzyme action for the pancreatic rennin is 60–65° C. (VERNON), which is much higher than for the trypsin, and GLAESSNER and POPPER [3] have also observed a case where the human pancreatic juice contained no rennin enzyme.

According to HALLIBURTON and BRODIE [4] casein is converted by the pancreatic juice of the dog into "pancreatic" casein, a substance which, in regard to solubility, stands to a certain extent between casein and paracasein (see Chapter XIV), and which is converted into paracasein by rennin. Further investigations on the action of this enzyme upon milk and especially upon pure casein solutions are very desirable.

The property of pancreatic juice of giving plastein precipitates is just as inexplicable as in the case of the gastric juice and other enzyme solutions.

Pancreatic Calculi. The concrement from a cystic enlargement of WIRSUNG'S duct in a man, as analyzed by BALDONI, contained in 1000 parts as follows: Water 34.4, ash 126.7, protein substances 34.9, free fatty acids 133, neutral fats 124, cholesterin 70.9, soaps and pigment 499.1, parts. SCHEUNERT and BERGHOLZ [5] have reported a pancreatic calculi in the ox.

Besides the enzymes which have been discussed in connection with the pancreatic juice, the gland also contains others, among which can be mentioned the enzyme which, according to STOKLASA and his collaborators, occurs chiefly in organs and tissues and which decomposes sugar into alcohol and carbon dioxide, like zymase. Opinions as to the importance of the pancreas for glycolysis are diverse, and we therefore

[1] Abderhalden with Koelker, Zeitschr. f. physiol. Chem., **54** and **55**; with Brahm, *ibid.*, **57**.

[2] Kühne and Roberts, Maly's Jahresber., **9**; see also Edkins, Journ. of Physiol., **12** (literature); Delezenne, Compt. rend. soc. biol., **62** and **63**; Wohlgemuth, Bioch. Zeitschr., **2**.

[3] Vernon, Journ. of Physiol., **12**; Glaessner and Popper, Deutsch. Arch. f. klin. Med., **94**.

[4] Journ. of Physiol., **20**.

[5] Baldoni, Maly's Jahresb., **29**, 353; Scheunert and Bergholz, Zeitschr. f. physiol. Chem., **52**.

refer the reader to what has been previously stated on this subject in Chapter VIII, pages 385 and 386.

V. THE CHEMICAL PROCESSES IN THE INTESTINE.

The action which belongs to each digestive secretion may be essentially changed under certain conditions by being mixed with other digestive fluids for various reasons, and also by the action of the enzymes upon each other;[1] and since the digestive fluids which flow into the intestine are mixed with still another fluid, the bile, it will be readily understood that the combined action of all these fluids in the intestine makes the chemical processes going on therein very complicated.

As the acid of the gastric juice acts destructively on ptyalin, this enzyme has no further diastatic action, even after the acid of the gastric juice has been neutralized in the intestine. ROGER and SIMON[2] claim to have observed in saliva made inactive by the gastric juice, a reactivation caused by the pancreatic juice, but these investigations do not seem to be fully conclusive. The bile has, at least in certain animals, a slight diastatic action, which in itself can hardly be of any great importance, but which shows that the bile has not a preventive, but rather a beneficial influence on the energetic diastatic action of the pancreatic juice. MARTIN, WILLIAMS, PAWLOW, and BRUNO[3] have observed a beneficial action of the bile on the diastatic action of the pancreas infusion. To this may be added that the organized ferments which habitually occur in the intestine and sometimes in the food have partly a diastatic action and partly produce a lactic-acid and butyric-acid fermentation. The maltose which is formed from the starch seems to be converted into dextrose in the intestine. Cane-sugar is inverted in the intestine, and, at least in certain animals, also lactose. There does not seem to be any doubt that cellulose, especially the fine and tender varieties, is in part dissolved in the intestine. LOHRISCH[4] found that on an average of 50 per cent of the introduced cellulose and hemicellulose was digested in human beings and yielded the corresponding sugar. That cellulose undergoes a fermentation in the intestine by the action of micro-organisms, producing marsh-gas, acetic acid, and butyric acid, has been especially shown by TAPPEINER; still it is not known to what extent the cellulose is destroyed in this way.[5]

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[1] See Wróblewski and collaborators, Hofmeister's Beiträge, **1**.

[2] Compt. rend. soc. biol., **62**.

[3] Martin and Williams, Proceed. of Roy. Soc., **45** and **48**; Bruno, footnote 2, p. 479.

[4] Cited from Bioch. Centralbl., 8, 334.

[5] On the digestion of cellulose see Henneberg and Stohmann, Zeitschr. f. Biologie, **21**, 613; v. Knieriem, *ibid.*, 67; Hofmeister, Arch. f. wiss. u. prakt. Thierheilkunde,

The bile has, as shown by MOORE and ROCKWOOD [1] and then especially by PFLÜGER, the property to a high degree of dissolving fatty acids, especially oleic acid, which itself is a solvent for other fatty acids, and hence, as will be seen later, it is of great importance in the absorption of fat. It is also of great importance that the bile, as previously stated, not only activates the steapsinogen, but that, as first shown by NENCKI and RACHFORD,[2] it accelerates the fat-splitting action of the steapsin. According to v. FÜRTH and SCHÜTZ [3] the bile-salts are the active constituents of the bile in this cleavage, and the fatty acids set free can combine with the alkalies of the intestinal and pancreatic juices and the bile, producing soaps which are of great importance in the emulsification of the fats.

If to a soda solution of about 1–3 p. m. pure, perfectly neutral olive-oil is added in not too large a quantity, a transient emulsion is obtained after vigorous shaking. If, on the contrary, one adds to the same quantity of soda solution an equal amount of commercial olive-oil (which always contains free fatty acids), the vessel need only be turned over for the two liquids to mix, and immediately there appears a very finely divided and permanent emulsion, making the liquid appear like milk. The free fatty acids of the commercial oil, which is always somewhat rancid, combine with the alkali to form soaps which act to emulsify the fats (BRÜCKE, GAD, LOEWENTHAL [4]). This emulsifying action of the fatty acids split off by the pancreatic juice is undoubtedly assisted by the habitual occurrence of free fatty acids in the food, as well as by the splitting off of fatty acids from the neutral fats in the stomach (see page 452).

Bile completely prevents peptic zymolysis in artificial digestion, because it retards the swelling up of the proteins. The passage of bile into the stomach during digestion, on the contrary, seems, according to several investigators, especially ODDI and DASTRE,[5] to have no disturbing action on gastric digestion. According to BOLDIREFF,[6] after continuous starvation, on feeding fat and food rich in fat, as well as after

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11; Weiske, Zeitschr. f. Biologie, **22**, 373; Tappeiner, *ibid.*, **20** and **24**; Mallèvre, Pflüger's Arch., **49**; Omeliansky, Arch. d. scienc. biol. de St. Pétersbourg, **7**; E. Müller, Pflüger's Arch., **83**; Lohrisch, Zeitschr. f. physiol. Chem., **47** (literature); and l. c., footnote 4, p. 488.

[1] Proceedings of Roy. Soc., **60**, and Journ. of Physiol., **21**. In regard to Pflüger's work see Absorption.

[2] Nencki, Arch. f. exp. Path. u. Pharm., **20**; Rachford, Journal of Physiol., **12**.

[3] Centralbl. f. Physiol., **20**.

[4] Brücke, Wien. Sitzungsber., **61**, Abt. 2; Gad, Arch. f. (Anat. u.) Physiol., 1878; Loewenthal, *ibid.*, 1897.

[5] Oddi, in Centralbl. f. Physiol., **1**, 312; Dastre, Arch. de Physiol. (5), **2**, 316.

[6] Centralbl. f. Physiol., **18**, 457, and Pflüger's Arch., **121**.

large amounts of acid, a mixture of bile, pancreatic juice, and intestinal juice pass readily into the stomach. After food rich in fat, which retards the secretion of gastric juice and the motility of the stomach, a digestion due to this alkaline mixture may take place in the stomach.

Bile itself has no solvent action on proteins in neutral or alkaline reaction, but still it may exert an influence on protein digestion in the intestine. The acid contents of the stomach, containing an abundance of proteins, give with the bile a precipitate of proteins and bile-acids. This precipitate carries a part of the pepsin with it, and for this reason, and also on account of the partial or complete neutralization of the acid of the gastric juice by the alkali of the bile and the pancreatic juice, the pepsin digestion cannot proceed further in the intestine. On the contrary, the bile does not disturb the digestion of proteins by the pancreatic juice in the intestine. The action of these digestive secretions, as above stated, is not disturbed by the bile, not even by the faintly acid reaction due to organic acids; but, on the contrary, the action of trypsin is accelerated by the bile. In a dog killed while digestion is going on, the faintly acid, bile-containing material of the intestine shows regularly a strong digestive action on proteins.

The precipitate formed on the meeting of the acid contents of the stomach with the bile easily redissolves in an excess of bile and also in the NaCl formed in the neutralization of the hydrochloric acid of the gastric juice. This may take place even in faintly acid reaction. Since in man the excretory ducts of the bile and the pancreatic juice open near one another, in consequence of which the acid contents of the stomach are probably immediately in great part neutralized by the bile as soon as it enters, it is doubtful whether a precipitation of proteins by the bile occurs in the intestine.

Besides the previously mentioned processes caused by enzymes, there are others of a different nature going on in the intestine, namely, the fermentation and putrefaction processes caused by microorganisms. These are less intense in the upper parts of the intestine, but increase in intensity toward the lower part, and decrease in the large intestine because of the consumption of fermentable material and by the removal of water by absorption. Fermentation processes, but only very slight putrefaction, occur in the small intestine of man. Macfadyen, M. Nencki, and N. Seeber[1] have investigated a case of human anus præternaturlis, in which the fistula occurred at the lower end of the ileum, and they were able to investigate the contents of the intestine after it had been exposed to the action of the mucous membrane of the entire small intestine. The mass was yellow or yellowish

[1] Arch. f. exp. Path. u. Pharm., 28.

brown, due to bilirubin, and had an acid reaction which, on a mixed but chiefly animal diet, calculated as acetic acid, amounted to 1 p. m. The contents were nearly odorless, having an empyreumatic odor recalling that of volatile fatty acids, and infrequently had a putrid odor resembling that of indol. The essential acid present was acetic acid, accompanied by fermentation and paralactic acid, volatile fatty acids, succinic acid, and bile-acids. Coagulable proteins, peptone, mucin, dextrin, dextrose, and alcohol were present. Leucine and tyrosine could not be detected.

According to the above-mentioned investigators, the proteins are only to a very slight extent, if at all, decomposed by the microbes in the small intestine of man. The organisms present in the small intestine preferably decompose the carbohydrates, forming ethyl alcohol and the above-mentioned organic acids.

Further investigations of JAKOWSKY and of AD. SCHMIDT[1] lead to the same result, namely, that in man the putrefaction of the proteins takes place chiefly in the large intestine, and the conditions are the same in carnivora. In these latter it has been possible to follow the intestinal digestion by investigating the contents of the various parts of the intestine as well as by forming fistulas along the intestine. Again PAWLOW and his pupils, especially LONDON[2] and his collaborators, have essentially advanced our knowledge on this subject.

In regard to the digestion of protein it has been found that after feeding meat, bread or certain protein bodies, the digestion in the stomach and small intestine is so complete that on the passage of the contents into the cæcum all the protein is digested and absorbed. Unboiled white of egg is an exception and is digested with difficulty. In experiments with unboiled white of egg LONDON and SULEIMA[3] reobtained 73 per cent of the coagulable protein from a fistula in the ileum (2–3 cm. in front of the cæcum). KUTSCHER and SEEMANN, ABDERHALDEN, LONDON and collaborators[4] have also found that non-biuret giving products and amino-acids are regularly split off, probably by the combined action of trypsin and erepsin. These amino-acids occur to a slight extent only, but from this no conclusion can be drawn as to the extent of amino-acid formation, because we do not know the extent of their absorption. The digestion of protein in the intestine,

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[1] Jakowsky, Arch. des scienc. biol. de St. Pétersbourg, **1**; Ad. Schmidt, Arch. f. Verdauunskr., **4**.

[2] The works of London and collaborators cannot be cited in detail, but may be found in Zeitschr. f. physiol. Chem., **46–57**.

[3] Zeitschr. f. physiol. Chem., **46**.

[4] Kutscher and Seemann, *ibid.*, **34**; Abderhalden and London, with Kautzsch, *ibid.*, with L. Baumann, *ibid.*, **51**, with v. Körösy, *ibid.*, **53**.

it seems, according to ABDERHALDEN, LONDON, OPPLER and REEMLIN,[1] is similar to the artificial digestion with trypsin, namely, that the destruction takes place step-wise, that certain amino-acids, such as tyrosine, are split off earlier than others.

The decomposition products of the proteins formed by the action of gastric juice can, according to LONDON,[2] be absorbed without further cleavage by the pancreatic juice, and a further cleavage in the intestine seems to be more necessary for assimilation than for absorption.

The carbohydrates and the fats (LEVITES[3]) may be so completely split in the stomach and small intestine that their absorption is complete before the contents pass into the cæcum. According to LONDON and POLOWZOWA[4] a strong cleavage of starch, dextrins and disaccharides takes place, especially in the duodenum, while the absorption is less strong here. The carbohydrates are here prepared for the absorption taking place in the lower parts of the intestine, though the cleavage also goes on in the other parts, namely in the jejunum and the upper part of the ileum.

As above remarked, ordinarily no putrefaction takes place in the small intestine, but occurs generally only in the large intestine. This putrefaction of the proteins is not the same as the pancreatic digestion. In putrefaction the decomposition goes much further and a mixture of products is obtained which have become known through the labors of numerous investigators, especially NENCKI, BAUMANN, BRIEGER, H. and E. SALKOWSKI, and their pupils. The products which are formed in the putrefaction of proteins are (in addition to *proteoses, peptones, amino-acids,* and *ammonia*) *indol, skatol, paracresol, phenol, phenylpropionic acid,* and *phenylacetic acid,* also *paraoxyphenylacetic acid* and *hydro-paracoumaric acid* (besides paracresol, produced in the putrefaction of tyrosin), *volatile fatty acids, carbon dioxide, hydrogen, marsh-gas, methyl-mercaptan,* and *sulphuretted hydrogen.* In the putrefaction of gelatin neither tyrosine nor indol is formed, while *glycocoll* is produced instead.

Among these products of decomposition a few are of special interest because of their behavior within the organism and because after their absorption they pass into the urine. A few, such as the oxyacids, pass unchanged into the urine. Others, such as phenols, are directly transformed into ethereal sulphuric acids by synthesis, and are eliminated as such by the urine; on the contrary, others, such as indol and skatol, are converted into ethereal sulphuric acids after oxidation only (for details see Chapter XV). The quantity of these bodies in the urine also varies with the extent of the putrefactive processes in the intestine; at least

[1] Zeitschr. f. physiol. Chem., 55 and 58.
[2] *Ibid.*, 49.
[3] Zeitschr. f. physiol. Chem., 49 and 53.
[4] *Ibid.*, 56.

this is true for the ethereal sulphuric acids. Their quantity increases in the urine with a stronger putrefaction, and the reverse takes place, namely, a disappearance from the urine, or a great reduction in quantity, as BAUMANN, HARLEY and GOODBODY [1] have shown by experiments on dogs, when the intestine is disinfected by various agents.

The *gases* which are produced by the decomposition processes are mixed in the intestinal tract with the atmospheric air swallowed with the saliva and food, and as the gas developed in the decomposition of different foods varies, so the mixture of gases after various foods should have a dissimilar composition. This is found to be true. *Oxygen* is found only in very faint traces in the intestine; this may be accounted for in part by the formation of reducing substances in the fermentation processes which combine with the oxygen, and partly, perhaps chiefly, to a diffusion of the oxygen through the tissues of the walls of the intestine. To show that these processes take place mainly in the stomach the reader is referred to page 461, on the composition of the gases of the stomach. *Nitrogen* is invariably found in the intestine, and it is probably due chiefly to the swallowed air. The *carbon dioxide* originates partly from the contents of the stomach, partly from the putrefaction of the proteins, partly from the lactic-acid and butyric-acid fermentation of carbohydrates, and partly from the setting free of carbon dioxide from the alkali carbonates of the pancreatic and intestinal juices by their neutralization through the hydrochloric acid of the gastric juice and by organic acids formed in the fermentation. *Hydrogen* occurs in largest quantities after a milk diet, and in smallest quantities after a purely meat diet. This gas seems to be formed chiefly in the butyric-acid fermentation of carbohydrates, although it may occur in large quantities in the putrefaction of proteins under certain circumstances. There is no doubt that the *methylmercaptan* and *sulphuretted hydrogen* which occur normally in the intestine originate from the proteins. The *marsh-gas* undoubtedly originates in the putrefaction of proteins. As proof of this RUGE [2] found 26.45 per cent marsh-gas in the human intestine after a meat diet. He found a still greater quantity of this gas after a vegetable (leguminous) diet; this coincides with the observation that marsh-gas may be produced by a fermentation of carbohydrates, but especially of cellulose (TAPPEINER [3]). Such an origin of marsh-gas, especially in herbivora, is to be expected. A small part of the marsh-gas and carbon dioxide may also arise from the decomposition of lecithin (HASEBROEK [4]).

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[1] Baumann, Zeitschr. f. physiol. Chem., **10**; Harley and Goodbody, Brit. Med. Journ., 1899.

[2] Wien. Sitzungsber., **44**.

[3] Zeitschr. f. Biologie, **20** and **24**.

[4] Zeitschr. f. physiol. Chem., **12**.

Putrefaction in the intestine not only depends upon the composition of the food, but also upon the albuminous secretions and the bile. Among the constituents of bile which are changed or decomposed there are not only the pigments—the bilirubin yields urobilin and a brown pigment—but also the bile-acids, especially taurocholic acid. Glycocholic acid is more stable, and a part is found unchanged in the excrement of certain animals, while taurocholic acid is so completely decomposed that it is entirely absent in the feces. In the fetus, on the contrary, in whose intestinal tract no putrefaction processes occur, undecomposed bile-acids and bile-pigments are found in the contents of the intestine. The transformation of bilirubin into urobilin does not occur, as previously stated, in the small, but in the large intestine in man.

As under normal conditions no putrefaction, or at least none worth mentioning, occurs in the small intestine, and as often nearly all the protein of the food is absorbed, it follows that ordinarily it is the secretions and cells rich in proteins which undergo putrefaction. That the secretions rich in proteins are destroyed in putrefaction in the intestine follows from the fact that putrefaction may also continue during complete fasting. From the observations of Müller[1] upon Cetti it was found that the elimination of indican during starvation rapidly decreased and after the third day of starvation it had entirely disappeared, while the phenol elimination, which at first decreased so that it was nearly minimum, increased again from the fifth day of starvation, and on the eighth or ninth day it was three to seven times as much as in man under ordinary circumstances. In dogs, on the contrary, the elimination of indican during starvation is considerable, but the phenol elimination is slight. Among the secretions which undergo putrefaction in the intestine, the pancreatic juice, which putrefies most readily, takes first place.

From the foregoing facts it must be concluded that the products formed by the putrefaction in the intestine are in part the same as those formed in digestion. The putrefaction may be of benefit to the organism in so far as the formation of such products as proteoses, peptones, polypeptides and amino-acids is concerned. The question has indeed been asked (Pasteur), is digestion possible without micro-organisms? Nuttal and Thierfelder have shown that guinea-pigs, removed from the uterus of the mother by Cæsarian section, could with sterile air digest well and assimilate sterile food (milk and crackers) in the complete absence of bacteria in the intestine, and developed normally and increased in weight. Schottelius[2] has arrived at other results by experiments with hens. The chickens, hatched under sterile conditions, kept in sterile rooms and

[1] Berlin. klin. Wochenschr., 1887.

[2] Nuttal and Thierfelder, Zeitschr. f. physiol. Chem., **21** and **22**, Schottelius, Arch. f. Hygiene, **34**, **42**, and **67**.

fed with sterile food, had continuous hunger and ate abundantly, but soon died, in about the same time as a starving chicken. On mixing with the food, at the proper time, a variety of bacteria from hen feces, they gained weight again and recovered.

The bacterial action in the intestinal canal is, at least in certain cases, necessary, and it acts in the interest of the organism. This action may, by the formation of further cleavage products, involve a loss of valuable material to the organism, and it is therefore important that putrefaction in the intestine be kept within certain limits. If an animal is killed while digestion in the intestine is going on, the contents of the small intestine give out a peculiar but not putrescent odor. Also the odor of the contents of the large intestine is far less offensive than a putrefying pancreas infusion or a putrefying mixture rich in protein. From this one may conclude that putrefaction in the intestine is ordinarily not nearly so intense as outside of the organism.

It seems thus to be provided, under physiological conditions, that putrefaction shall not proceed too far, and the factors which here come into consideration are probably of different kinds. Absorption is undoubtedly one of the most important of them, and it has been proven by actual observation that the putrefaction increases, as a rule, as the absorption is checked and fluid masses accumulate in the intestine. The character of the food also has an unmistakable influence, and it seems as if a large quantity of carbohydrates in the food acts against putrefaction (Hirschler [1]). It has been shown by Pöhl, Biernacki, Rovighi, Winternitz, Schmitz, and others [2] that milk and kephir have a specially strong preventive action on putrefaction. This action is not due to the casein, but chiefly to the lactose and also in part to the lactic acid.

A specially strong preventive action on putrefaction has been ascribed for a long time to the bile. This anti-putrid action does not exist in neutral or faintly alkaline bile, which itself easily putrefies, but to the free bile-acids, especially taurocholic acid (Maly and Emich, Lindberger [3]). There is no question that the free bile-acids have a strong preventive action on putrefaction outside of the organism, and it is therefore difficult to deny such an action in the intestine. Notwithstanding this, the anti-putrid action of the bile in the intestine is not considered by certain investigators (Voit, Röhmann, Hirschler and Terray, Landauer and Rosenberg [4]) as of great importance.

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[1] Hirschler, Zeitschr. f. physiol. Chem., **10**; Zimnitzki, *ibid.*, **39** (literature).

[2] Schmitz, *ibid.*, **17**, 401, which gives references to the older literature, and **19**. See also Salkowski, Centralbl. f. d. med. Wiss., 1893, 467, and Seelig, Virchow's Arch., **126** (literature).

[3] Maly and Emich, Monatshefte f. Chem., **4**; Lindberger, footnote 3, p. 483.

[4] Voit, Beitr. zur Biologie, Jubiläumschrift, Stuttgart, 1882; Röhmann, Pflüger's

Biliary fistulas have been established so as to study the importance of the bile in digestion (SCHWANN, BLONDLOT, BIDDER and SCHMIDT,[1] and others). As a result it has been observed that with fatty foods an imperfect absorption of fat regularly takes place and the excrement contains, therefore, an excess of fat and have a light-gray or pale color. The extent of deviation from the normal after the operation is essentially dependent upon the character of the food. If an animal is fed on meat and fat, then the quantity of food must be considerably increased after the operation, otherwise the animal will become very thin, and indeed die with symptoms of starvation. In these cases the excrement has the odor of carrion, and this was considered a proof of the action of the bile in checking putrefaction. The emaciation and the increased want of food depend, naturally, upon the imperfect absorption of the fats, whose high calorific value is reduced and must be replaced by the taking up of larger quantities of other nutritive bodies. If the quantity of proteins and fats be increased, then the latter, which can be only incompletely absorbed, accumulate in the intestine. This accumulation of the fats in the intestine only renders the action of the digestive juices on proteins more difficult, and thus increases the amount of putrefaction. This explains the appearance of fetid feces, whose pale color is not due to a lack of bile-pigments, but to a surplus of fat (RÖHMANN, VOIT). If the animal is, on the contrary, fed on meat and carbohydrates, it may remain quite normal, and the leading off of the bile does not cause any increased putrefaction. The carbohydrates may be uninterrupedly absorbed in such large quantities that they replace the fat of the food, and this is the reason why the animal on such a diet does not become emaciated. As with this diet the putrefaction in the intestine is no greater than under normal conditions even though the bile is absent, it would seem that the bile in the intestine exercises no preventive action on putrefaction.

To this conclusion the objection may be made that the carbohydrates, which are capable of checking putrefaction, can, so to speak, undertake the anti-putrid action of the bile. But as there are also cases (in dogs with biliary fistula) where the intestinal putrefaction is not increased with exclusive meat diet,[2] it is maintained that the absence of bile in the intestine, even by exclusive carbohydrate food, does not always cause an increased putrefaction.

Although the question as to the manner in which the putrefactive

Arch. 29; Hirschler and Terray, Maly's Jahresber., 26; Landauer, Math. u. Naturw. Ber aus Ungarn, 15; Rosenberg, Arch. f. (Anat. u.) Physiol., 1901.

[1] Schwann, Müller's Arch. f. Anat. u. Physiol., 1844; Blondlot, cited from Bidder and Schmidt, Verdauungssäfte, etc., 98.

[2] See Hirschler and Terray, l. c.

processes in the intestine under physiological conditions are kept within certain limits cannot be answered positively, still it may be asserted that the faint acid reaction and the absorption of water and the relatively rapid movement, of the contents of the small intestine and its absorption, are important factors.

That the acid reaction in the intestine has a preventive influence on the putrefactive processes follows from the existing relation between the degree of acidity of the gastric juice and the putrefaction in the intestine. Since the investigations and observations of KAST, STADELMANN, WASBUTZKI, BIERNACKI and MESTER had proven that an increased putrefaction in the intestine occurred when the quantity of hydrochloric acid in the gastric juice was diminished or deficient, SCHMITZ [1] has lately shown in man that on the administration of hydrochloric acid, producing a hyperacidity of the gastric juice, the putrefaction in the intestine may be checked. The question arises whether the reaction in the small intestine is always acid and whether the acidity is strong enough to prevent putrefaction. In this connection it must be recalled that the acidity of the contents of the small intestine is not due to hydrochloric acid, but chiefly to organic acids, acid salts, and free carbon dioxide. There are several observations as to the reaction of the intestinal contents, by MOORE and ROCKWOOD, MOORE and BERGIN, MATTHES and MARQUARDSEN, I. MUNK, NENCKI and ZALESKI, HEMMETER,[2] although they are somewhat contradictory. From these reports one can conclude that the reaction may vary not only among different animals, but also in the same animals under varying conditions. There is no doubt that the acid reaction in many cases is due to the presence of organic acids. On testing with various indicators it has been shown that sometimes the upper parts, and often the lower parts, are acid, due to acid salts such as $NaHCO_3$ and free CO_2, and finally that in certain animals the intestinal contents are alkaline throughout. The question how, under these conditions, putrefaction is excluded, and how the acidity of the gastric contents influences the intestinal putrefaction, cannot be explained. It is very probable that the bacterial flora of the intestine is of very great importance and it is possible, as BIENSTOCK admits, that the explanation lies in an antagonistic bacterial action and that the carbohydrates, especially lactose, which retard putrefaction, form a good nutritive media for those bacteria which destroy the putrefactive producers or retard their development. According to HOROWITZ an unequal

[1] Zeitschr. f. physiol. Chem., **19**, 401, which includes all the pertinent literature.

[2] Moore and Rockwood, Journ. of Physiol., **21**; Moore and Bergin, Amer. Journ. of Physiol., **3**; Matthes and Marquardsen, Maly's Jahresber., **28**; Munk, Centralbl. f. Physiol., **16**; Nencki and Zaleski, Zeitschr. f. physiol. Chem., **27**; Hemmeter, Pflüger's Arch., **81**.

division of the various bacteria occurs in dogs in the different parts of the intestine and certain varieties of bacteria occur in greater quantities than others, according to the kind of food taken. Perhaps, also, agreeing with the experience of CONRADI and KURPJUWEIT,[1] the toxines produced by the intestinal bacteria may, by their antiseptic action, keep the putrefactive processes in the intestine within bounds.

Feces. It is evident that the residue which remains after complete digestion and absorption in the intestine must be different, both qualitatively and quantitatively, according to the variety and quantity of the food. In man the quantity of excrement from a mixed diet is 120–150 grams, with 30–37 grams of solids, per twenty-four hours, while the quantity from a vegetable diet, according to VOIT,[2] was 333 grams, with 75 grams of solids. With a strictly meat diet the excrement is scanty, pitch-like, and black. The scanty feces in starvation has a similar appearance. A large quantity of coarse bread yields a great amount of light-colored excrement. In these cases the feces are also habitually poorer in nitrogen than after food rich in protein. The individuality also plays an important rôle in the utility of the food and the formation of feces (SCHIERBECK[3]). If there is a large proportion of fat, it takes a lighter clay-like appearance. The decomposition products of the bile-pigments seem to play only a small part in the normal color of the feces.

The constituents of the feces are of different kinds. In the excrement are found digestible or absorbable constituents of the food, such as muscle fibres, connective tissues, lumps of casein, grains of starch, and fat, which have not had sufficient time to be completely digested or absorbed in the intestinal tract. In addition the excrement contains indigestible bodies, such as the remains of plants, keratin substances, and others; also form-elements originating from the mucous coat and the glands; constituents of the different secretions, such as mucin, cholic acid, dyslysine, and cholesterin (koprosterin or stercorin), purine bases,[4] and enzymes; mineral bodies of the food and the secretions; and, lastly, products of putrefaction or of digestion, such as skatol, indol, volatile fatty acids, purine bases, lime, and magnesia soaps. Occasionally, also, parasites of different kinds occur; and lastly, the excrement contains micro-organisms of various species.

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[1] Bienstock, Arch. f. Hygiene, **39**; Horowitz, Zeitschr. f. physiol. Chem., **52**; Conradi and Kurpjuweit, Münch. med. Wochenschr., 1905.

[2] Zeitschr. f. Biologie, **25**, 264.

[3] Arch. f. Hygiene, **51**.

[4] In regard to the purine bases in feces, see Hall, Journ. of Path. and Bacteriol., **9**; Schittenhelm, Arch. f. klin. Med., **81**; Schittenhelm and Krüger, Zeitschr. f. physiol. Chem., **45**.

That the mucous membrane of the intestine by its secretion and by the abundant quantity of detached epithelium contributes essentially to the formation of feces follows from the discovery first made by L. HERMANN and substantiated by others,[1] that a clean, isolated loop of intestine collects material similar to feces. These masses are rich in mineral substances and especially rich in bodies soluble in alcohol-ether, among which cholesterin occurs, as previously mentioned (Chapter VIII). With a mixed diet with an excess of meat, the human feces consist only in small part of food residues and consist in great part, or after meat or milk diet, nearly entirely, of intestinal secretions. Many foods, therefore, produce a large quantity of feces chiefly by causing an abundant secretion.[2]

The reaction of the feces is very variable, but in man with a mixed diet it is neutral or faintly alkaline. It is often acid in the inner part, while the outer layers in contact with the mucous coat have an alkaline reaction. In nursing infants it is habitually acid. The odor is perhaps chiefly due to skatol, which was first found in the feces by BRIEGER, and so named by him. Indol and other substances also take part in the production of odor. The color is ordinarily light or dark brown, and depends above all upon the nature of the food. Medicinal bodies may give the feces an abnormal color. The excrement is colored black by bismuth, yellow by rhubarb, and green by calomel. This last-mentioned color was formerly accounted for by the formation of a little mercury sulphide, but now it is said that calomel checks the putrefaction and the decomposition of the bile-pigments, so that a part of the bile-pigments passes into the feces as biliverdin. In the yolk-yellow or greenish-yellow excrement of nursing infants one can detect bilirubin. Neither bilirubin nor biliverdin seems to exist in the excrement of mature persons under normal conditions. On the contrary, there is found *stercobilin* (MASIUS and VANLAIR), which is identical with urobilin (JAFFÉ [3]). Bilirubin may occur in pathological cases in the feces of mature persons. It has been observed in a crystallized state (as hæmatoidin) in the feces of children as well as of grown persons.

The absence of bile (acholic feces) causes the feces to have, as above stated, a gray color, due to large quantities of fat; this may, however, be partly attributed to the absence of bile-pigments. In these

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[1] Hermann, Pflüger's Arch., **46**. See also Ehrenthal, *ibid.*, **48**; Berenstein, *ibid.*, **53**; Klecki, Centralbl. f. Physiol., **7**; 736, and F. Voit, Zeitschr. f. Biologie, **29**; v. Moraczewski, Zeitschr. f. physiol. Chem., **25**; F. Lippich, Prager med. Wochenschr., **32**.

[2] In regard to the constitution of feces with various foods, see Hammerl, Kermauner, Moeller, and Prausnitz, Zeitschr. f. Biologie, **35**, and Pöda, Micko, Prausnitz and Müller, *ibid* , **39**.

[3] See bile-pigments, Chapter VIII, and urobilin, Chapter XV.

cases a large quantity of crystals has been observed which consist chiefly of magnesium soaps or sodium soaps. Hemmorrhage in the upper parts of the digestive tract yields, when it is not very abundant, a dark-brown excrement, due to hæmatin.

EXCRETIN, so named by MARCET,[1] is a crystalline body occurring in human excrement, but which, according to HOPPE-SEYLER, is perhaps only impure cholesterin (koprosterin or stercorin?). EXCRETOLIC ACID is the name given by MARCET to an oily body with an excrementitious odor.

In consideration of the very variable composition of feces, quantitative analyses are of little value and therefore will be omitted.[2]

Meconium is a dark brownish-green, pitchy, mostly acid mass without any strong odor. It contains greenish-colored epithelium cells, cell-detritus, numerous fat-globules, and cholesterin plates. The amount of water is 720–800, and solids 280–200 p. m. Among the solids there are mucin, bile-pigments, and bile-acids, cholesterin, fat, soaps, traces of enzymes, calcium and magnesium phosphates. Sugar and lactic acid, soluble protein bodies and peptones, also leucine and tyrosine and the other products of putrefaction occurring in the intestine, are absent. Meconium may contain undecomposed taurocholic acid, bilirubin and biliverdin, but it does not contain any stercobilin, which is considered as proof of the non-existence of putrefactive processes in the digestive tract of the fetus.

The contents of the intestine under abnormal conditions are perhaps less the subject of chemical analysis than of an inspection and microscopical investigation or bacteriological examination. On this account the question as to the properties of the contents of the intestine in different disease cannot be thoroughly treated here.[3]

Appendix.

INTESTINAL CONCREMENTS.

Calculi occur very seldom in the human intestine or in the intestine of carnivora, but they are quite common in herbivora. Foreign bodies or undigested residues of food may, when for some reason or other they are retained in the intestine for some time, become incrusted with salts, especially ammonium-magnesium phosphate or magnesium phosphate, and these salts usually form the chief constituent of the concrements. In man they are sometimes oval or round, yellow, yellowish gray, or brownish gray, of variable size, consisting of concentric layers and containing chiefly ammonium-magnesium phosphate and calcium phosphate, besides a small quantity of fat or pigment. The nucleus ordinarily consists of some foreign body, such as the stone of a fruit, a fragment of bone, or something similar. In those countries where bread made from oat-

[1] Annal. de chim. et de phys., 59.

[2] In regard to these analyses as well as to the feces under abnormal conditions and to the pertinent literature, see Ad. Schmidt and J. Strassburger, Die Fæces des Menschen, etc., Berlin, 1901 and 1902.

[3] See Schmidt and Strassburger, l. c.

bran is an important food, we often find in the large intestine balls similar to the so-called hair-balls (see below). Such calculi contain calcium and magnesium phosphate (about 70 per cent), oat-bran (15–18 per cent), soaps and fat (about 10 per cent). Concretions which contain very much fat (about 74 per cent) occasionally occur, and those consisting of fibrin clots, sinews, or pieces of meat incrusted with phosphates are also rare.

Intestinal calculi often occur in animals, especially in horses fed on bran. These calculi, which attain a very large size, are hard and heavy (as much as 8 kilos) and consist in great part of concentric layers of ammonium-magnesium phosphate. Another variety of concrements which occur in horses and cattle consists of gray-colored, often very large, but relatively light stones which contain plant residues and earthy phosphates. Stones of a third variety are sometimes cylindrical, sometimes spherical, smooth, shining, brownish on the surface, consisting of matted hairs and plant-fibres, and termed *hair-balls*. The so-called "ÆGAGROPILÆ," which occur in the ANTILOPUS RUPICAPRA, belong to this group, and are generally considered as nothing else than the hair-balls of cattle.

The so-called *oriental bezoar-stone* also belongs to the intestinal concrements, and probably originates from the intestinal tract of the CAPRA ÆGAGRUS and ANTELOPE DORCAS. There may exist two varieties of bezoar-stones. One is olive-green, faintly shining and formed of concentric layers. On heating it melts with the development of an aromatic odor. It contains as chief constituent LITHOFELLIC ACID, $C_{20}H_{36}O_4$, which is related to cholic acid, and besides this a bile-acid, LITHOBILIC ACID. The others are nearly blackish brown or dark green, very glossy, consisting of concentric layers, and do not melt on heating. They contain as chief constituent ellagic acid, a derivative of gallic acid, of the formula $C_{14}H_6O_8$, which, according to GRAEBE,[1] is the dilactone of hexaoxydiphenyldicarboxylic acid, and which gives a deep-blue color with an alcoholic solution of ferric chloride. The last-mentioned bezoar-stone originates, to all appearances, from the food of the animal.

Ambergris is generally considered an intestinal concrement of the sperm whale. Its chief constituent is *ambrain*, which is a non-nitrogenous substance perhaps related to cholesterin. Ambrain is insoluble in water and is not changed by boiling alkalies. It dissolves in alcohol, ether, and oils.

VI. ABSORPTION.

The problem of digestion consists in part in separating the valuable constituents of the food from the useless ones and dissolving or transforming them into forms which are adapted for the processes of absorption. In discussing the absorption processes we must treat of the form into which the different foods are changed before absorption, of the manner in which this is accomplished, and lastly, of the forces which act in these processes.

Before we can answer the question as to the form in which the proteins are absorbed from the intestinal canal, it is of interest to learn whether the animal body can, perhaps, also utilize such protein as are introduced intravenously, subcutaneously, or into a body-cavity, i.e., evading the intestinal canal, or, as OPPENHEIMER calls it, parenteral.

[1] Ber. d. d. chem. Gesellsch., **36**.

Since the first investigations of ZUNTZ and v. MERING on this subject several experimenters, such as NEUMEISTER, FRIEDENTHAL and LEWANDOWSKY, MUNK and LEWANDOWSKY, OPPENHEIMER, MENDEL, and ROCKWOOD, HEILNER, CRAMER and PRINGLE, MICHAELIS and RONA and others,[1] have shown, without any doubt, that the animal body can more or less completely utilize different, parenterally introduced proteins, although different varieties of animals show a difference in this regard. Still we do not know where and how these foreign proteins are changed and assimilated, but CRAMER ascribes great importance to the leucocytes in this regard.

That the animal body can also assimilate not previously digested or split proteins introduced directly into the intestine has been shown by BRÜCKE, BAUER and VOIT, EICHHORST, CZERNY and LATSCHENBERGER, VOIT and FRIEDLÄNDER, and others.[2] In the experiments of the two last-mentioned investigators neither casein (as milk) nor hydrochloric-acid myosin or acid albuminate (in acid solution) was absorbed, while, on the contrary, about 21 per cent of ovalbumin or seralbumin and 69 per cent of alkali albuminate (dissolved in alkali) were absorbed. MENDEL and ROCKWOOD, on the contrary, in experiments with casein and edestin in the living intestinal loop, could prove only the slightest absorption on excluding digestion as completely as possible, while the corresponding proteoses were abundantly absorbed.

It is difficult to decide in these experiments as to how far the proteins were taken up in an actually unchanged or partly modified form. The alimentary albuminaria, observed repeatedly after the introduction of large quantities of protein into the intestinal canal, indicates an absorption of undigested protein under certain circumstances. To decide this question the biological method, using the precipitine reaction, has been made use of, and ASCOLI and VIGNO,[3] using this method, claim to have shown the passage of non-modified protein into the blood and lymph. Based upon many investigations on this subject we can consider it possible that under certain circumstances, as on flooding the intestinal canal with protein,

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[1] Zuntz and v. Mering, Pflüger's Arch., **32**; Neumeister, Verh. d. phys.-med. Gesellsch. zu Würzburg, 1889, and Zeitschr., f. Biologie, **27**; Friedenthal and Lewandowsky, Arch. f. (Anat. u.) Physiol., 1899; Munk and Lewandowsky, *ibid.*, 1899, Supp.; Oppenheimer, Hofmeister's Beiträge, **4**; Mendel and Rockwood, Amer. Journ. of Physiol., **12**: Heilner, Zeitschr. f. Biol., **50**, and Münch. med. Wochenschr., **49**; Cramer, Journ. of Physiol., **37**, with Pringle, *ibid.*; Rona and Michaelis, Pflüger's Arch., **123** and **124**.

[2] Brücke, Wien. Sitzungsber., **59**; Bauer and Voit, Zeitschr. f. Biologie, **5**; Eichhorst, Pflüger's Arch., **4**; Czerny and Latschenberger, Virchow's Arch., **59**; Voit and Friedländer, Zeitschr. f. Biologie, **32**. Contradictory observations can be found in Keller, Beitr. z. Frage d. Resorption im Dickdarm. Inaug.-Dissert. Breslau, 1909.

[3] Zeitschr. f. physiol. Chem., **39**.

with a greater permeability of the intestinal wall, as in new-born and sucking animals, and with a diminished modification by the gastric juice, a passage of non-modified protein may take place in the blood-vessels, but that under normal conditions this is not the case, or at least does not take place to any mentionable degree. As a rule, the absorption of protein follows a modification of it, and the next question is whether the proteins are chiefly absorbed as proteoses or peptones or as simpler atomic complexes.

According to the earlier investigations of Ludwig and Schmidt-Mülheim as well as those of Munk and Rosenstein [1] it is generally agreed that the products of protein digestion do not pass into the blood through the lymph vessels, but through the intestinal capillaries. The question of the absorption of these products resolves itself into the form in which they are taken up by the intestine and the form in which they pass into the blood.

It was mentioned above that proteoses and peptones as well as non-biuret-giving products and amino-acids have been found in the contents of the intestine. The amino-acids occur to a less extent than the proteoses and peptones. This may indicate that the amino-acids are more abundantly formed, but also more quickly absorbed, but it may also indicate that the amino-acids are produced to a slight extent only, in the intestinal contents. There is no doubt that the amino-acids can be absorbed as such, but there is still another question, namely, whether the proteoses and peptones are absorbed as such or only after a previous cleavage into amino-acids.

Nolf and Honoré found, what was later substantiated by Zunz,[2] that the proteoses and peptones disappear more quickly from the intestine than the non-biuret-giving products. This does not prove that the proteoses are absorbed as such, but rather against such a view. A more direct proof for the absorption of the non-split proteoses lies in the fact, as shown by Nolf, that the proteoses when introduced in large quantities in the intestine pass in small amounts into the blood. Another proof is the findings of Borchardt [3] that after feeding dogs with not too large amounts of elastin, the passage of a proteose, the hemielastose, could be detected in the blood. Attention must also be called to the fact that according to Hofmeister [4] the walls of the

[1] Schmidt-Mülheim, Arch. f. (Anat. u.) Physiol., 1877; Munk and Rosenstein, Virchow's Arch., **123**.

[2] Nolf and Honoré, Arch. internat. de Physiol., 1905; Nolf, Journ. de Physiol. et Pathol. gén., 1907; Zunz, Mémoires cour., etc., Acad. Roy. Med., Belg., **20**, Fasc. 1.

[3] In regard to the literature on proteoses in the blood see Chapter VI, footnotes 3 and 4, p. 255, and 1, p. 256.

[4] Zeitschr. f. physiol. Chem., **6**, and Arch. f. exp. Path. u. Pharm., **19**, **20**, **22**.

stomach and intestine are the only parts of the body in which proteoses and peptones occur during digestion.

We have reason for believing that the proteoses, as well as their cleavage products, are taken up by the intestine, and if this is the case the next question to be answered is, in what form do these bodies leave the intestine and pass into the blood?

In order to decide this question the blood has been repeatedly tested in regard to the quantity of proteoses. As seen on pages 255 and 256 this has led to very contradictory results, and if we exclude those exceptional cases where a large quantity of proteose was introduced into the intestine at once, then we can say that the occurrence of proteoses in the blood, or at least in the blood plasma, has not been positively shown under physiological conditions.[1] It can also be said that such investigations do not prove much because of the large quantity of blood passing through the intestine for a given time, and the quantity of proteose must be so small so that when divided in the entire blood it can hardly be detected. It is therefore of interest that neither amino-acids nor proteoses were found in the blood after cutting out several organs or groups of organs so that the blood circulated only through the intestinal canal, heart, lungs, pancreas and intercostal muscles (KUTSCHER and SEEMANN, v. KÖRÖSY).[2]

We are therefore obliged to consider that the proteoses and amino-acids are transformed in the intestinal walls in some manner or other. Such a belief, especially applied to the proteoses, coincides with the observations of HOFMEISTER that the proteoses occurring in the mucous membrane during digestion disappear at the temperature of the room from the removed, but still apparently living, mucous membrane after a certain time. This also coincides well with the observations of LUDWIG and SALVIOLI.[3] These investigators introduced a peptone solution into a double-ligatured, isolated piece of the small intestine, which was kept alive by passing defibrinated blood through it, and observed that the peptone disappeared from the intestine, but that the blood passing through did not contain any peptone.

What becomes of the amino-acids in the intestinal wall? KUTSCHER and SEEMANN have shown that the crystalline cleavage products are so transformed in the intestinal wall that they cannot be detected. We have here to think of two possibilities: The amino-acids are either further split or they are used in synthesis (of proteins?)

It is a long-known fact that with the digestion and absorption an

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[1] See foot-note 3, p. 503.

[2] Kutscher and Seemann, Zeitschr. f. physiol. Chem., **34**; v. Körösy, *ibid.*, **57**.

[3] Arch. f. (Anat. u.) Physiol., 1880, Supplbd. See also Cathcart and Leathes, Journ. of Physiol., **33**.

increased elimination of nitrogen in the urine goes hand in hand. The quantity of nitrogen eliminated in the urine after partaking of protein corresponded, according to ASHER and HAAS,[1] to 65 per cent of the nitrogen introduced. It is hardly credible that this elimination of nitrogen depends upon an increased destruction of body protein, and it is more probable that this represents decomposed food-protein. But according to NENCKI and ZALESKI[2] an abundant formation of ammonia occurs in the cells of the digestive apparatus after a rich protein diet, so we must consider the possibility that a considerable part, perhaps the very greatest part, of the amino-acids are deamidized in the intestinal wall. The other part of the amino-acids may be used in the syntheses to be mentioned below. Such a partial deamidization of the digestive products has been shown by COHNHEIM[3] in his absorption experiments with the fish intestine.

The proteoses taken up by the intestinal mucosa, if this does take place, can naturally undergo a further conversion into amino-acids in the walls of the intestine. Still there are other possibilities. A direct utilization of the proteoses in the synthesis of the proteins in the intestine is not very probable, but on the contrary it is more probable that the proteoses, in order to undergo further cleavage or further utilization, are taken up by the leucocytes and carried off. HOFMEISTER has advocated such a possibility for a long time. HEIDENHAIN raised objections to this suggestion in which he called attention to the disproportion between the number of leucocytes and the large quantity of peptones (proteoses) to be absorbed, but at that time the deep cleavage of a great part of the protein into amino-acids was not known. Recently PRINGLE and CRAMER[4] urged the theory of the importance of the leucocytes, and the observations of INAGAKI[5] also show the possibility of the leucocytes taking up the proteoses and fixing them, it seems, in the cell substance.

It is for the present impossible to say with certainty whether or not and to what extent the proteoses, as such, are absorbed and to give their further fate thereafter in the intestine. The present view is probably as follows: That they do not pass as such into the blood, and that they are transformed into amino-acids in part in the intestinal contents and in part in the intestinal mucosa, and then from these amino-acids

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[1] Bioch. Zeitschr., **12**.

[2] Arch. des scienc. biol. de St. Pétersbourg, **4**; Arch. f. exp. Path. u. Pharm., **37**; see also Salaskin, Zeitschr. f. physiol. Chem., **25**.

[3] Zeitschr. f. physiol. Chem., **59**.

[4] Hofmeister, l. c.; Heidenhain, Pflüger's Arch., **43**; Pringle and Cramer, Journ. of Physiol., **37**.

[5] Zeitschr. f. physiol. Chem., **50**.

the coagulable proteins are constructed by synthesis. In support of the theory of a protein synthesis from amino-acids we have a series of experiments where deeply split or completely split proteins were fed. In these experiments by Loewi, Henderson and Dean, Henriques and Hansen, and especially by Abderhalden and his co-workers [1] on dogs, mice and rats, it was possible to keep the animals in nitrogenous equilibrium or indeed nitrogen retention for a long time with the cleavage products of proteins besides non-nitrogenous food-stuffs and salts. Especially important are certain experiments of Abderhalden and Rona and Abderhalden with Olinger, Messner and Windrath with completely decomposed meat.

The results of the experiments are generally considered as proof of the ability of the animal body to construct proteins from amino-acids by synthesis, and in the present state of our knowledge we can hardly draw other conclusions from them or advance any simpler theory. It is nevertheless true that a nitrogen retention is not synonymous with a new formation of protein, and we must not overlook the fact that Lüthje [2] observed a nitrogen retention on feeding with one amino-acid and abundance of carbohydrate, a condition where we can hardly speak of a synthesis of protein. Still these experiments do not seem to be conclusive enough to discard the above-mentioned experiments and for the present, as above stated, we must admit that these experiments give proof as to the synthesis of protein. With this we naturally do not explain the occurrence or the extent of such a synthesis under normal conditions.

Where does the protein synthesis take place? If it were positively sure that the amino-acids did not pass into the blood then we would have transferred this synthesis to the intestinal walls. Otherwise we must think in the first place of the liver; but this organ does not seem to play an important rôle in this synthesis. Abderhalden and London [3] made an experiment on a dog with an Eck fistula (see page 376), feeding the dog with decomposed protein, and they found that this animal behaved exactly like a normal animal, as it was kept for eight days not only in nitrogenous equibrilium but also in nitrogen retention. According to them the liver has no special function in the protein synthesis and the theory is more reasonable that the protein synthesis takes place in the intestine.

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[1] Loewi, Arch. f. exp. Path. u. Pharm., 48. See also Henderson and Dean, Amer. Journ. of Physiol., 9; Abderhalden and Rona, Zeitschr. f. physiol. Chem., 42, 44, 47, and 52; Henriques and Hansen, *ibid.*, 43, 49; Henriques, *ibid.*, 54; Abderhalden with Olinger, *ibid.*, 57, with Messner and Windrath, *ibid.*, 59.

[2] Pflüger's Arch., 113.

[3] Zeitschr. f. physiol. Chem., 54.

What kind of protein is formed in the synthesis? This we do not know. ABDERHALDEN'S belief is that it is plasma protein, which, as is well known, is the same in each animal independent of the kind of protein introduced with the food from which the cells of the body then create the further protein material. Objections can be raised against this hypothesis, but still it is worth consideration. In favor of this we can also add that according to the investigations of FREUND and v. KÖRÖSY [1] the blood coming from the intestine during digestion is richer in coagulable protein than other blood, and also that this protein FREUND asserts, belongs to the globulin group. This globulin, according to FREUND and TOEPFER, is not identical with the ordinary serglobulin mixture, but is a pseudoglobulin formed in the intestine from the food protein by synthesis, and which is more easily decomposed or further utilized in the liver and other organs. Further research in this direction is necessary, as we have other investigations which are essentially different. If a re-formation of coagulable proteins takes place from amino-acids during digestion, it is to be expected that a relatively greater quantity of coagulable protein should occur in the mucosa of the digesting intestine as compared with the non-digesting intestine. PRINGLE and CRAMER, by a method [1] which requires confirmation, claim that in the digesting animal (cat) the blood, and to a still higher degree the intestinal mucosa, and especially the lymph nodes of the intestine, are richer in non-coagulable protein than the starving animal, a condition which is related to the rôle of the leucocytes in the protein assimilation. This question of the absorption of proteins in the intestine is still unexplained in many directions.

The extent of the protein absorption is dependent essentially upon the kind of food introduced, since as a rule the protein substances from an animal source are much more completely absorbed than from a vegetable source. As proof of this the following observations are given: In his experiments on the utilization of certain foods in the intestinal canal of man RUBNER found that with an exclusively animal diet, on partaking of an average of 738–884 grams of fried meat or 948 grams of eggs per day, the nitrogen deficit with the excrement was only 2.5–2.8 per cent of the total nitrogen introduced. With a strictly milk diet the results were somewhat unfavorable, since after partaking of 4100 grams of milk the nitrogen deficit increased to 12 per cent. The conditions are quite different with vegetable food, as shown by the researches of MEYER, RUBNER, HULTGREN and LANDERGREN, who made experiments with various kinds of rye bread and found that the loss of nitro-

[1] v. Körösy, Zeitschr. f. physiol. Chem., **57**; Freund, Zeitschr. f. exp. Path. u. Therap., **4**; G. Toepfer and Freund, and Toepfer, *ibid.*, **3**; Pringle and Cramer, Journ. of Physiol., **37**.

gen through the feces amounted to 22–48 per cent. Experiments with other vegetable foods, and also the investigations of SCHUSTER, CRAMER, MEINERT, MORI,[1] and others on the utilization of foods with mixed diets, have led to similar results. With the exception of rice, wheat bread, and certain very finely divided vegetable foods, it is found in general that the nitrogen deficit by the feces increases with a larger quantity of vegetable material in the food.

The reason for this is manifold. The large quantity of cellulose frequently present in vegetable foods impedes the absorption of proteins. The greater irritation produced by the vegetable food itself or by the organic acids formed in the fermentation in the intestinal canal causes a more violent peristalsis, which drives the contents of the intestine faster than otherwise along the intestinal canal. Another and most important reason is the fact that a part of the vegetable protein substances seem to be indigestible, and because of the difficultly digestible vegetable food, a large quantity of digestion fluids containing nitrogen are secreted.

In speaking of the functions of the stomach we stated that after the removal or excision of this organ, an abundant digestion and absorption of proteins may take place. It is therefore of interest to learn how the digestion and absorption of proteins go on after the extirpation of the second protein-digesting organ, the pancreas. In this connection there are the observations on animals after complete or partial extirpation of the gland by MINKOWSKI and ABELMANN, SANDMEYER, V. HARLEY, after destroying the gland by ROSENBERG, and also in man after closing the pancreatic duct by HARLEY and DEUCHER. In all these cases such discrepancy of figures has resulted for the utilization of the proteins—between 80 per cent after the apparently complete exclusion of pancreatic juice in man (DEUCHER) and 18 per cent after extirpation of the gland in dogs (HARLEY)—that one can hardly draw any clear conception as to the extent and importance of the trypsin digestion in the intestine. That on completely preventing the entrance of pancreatic juice only a slight diminution in the protein absorption takes place follows from the researches of LOMBROSO and NIEMANN.[2] In order to understand in these cases why the digestion and absorption took place so abundantly it would be of interest to know how other diges-

[1] Rubner, Zeitschr. f. Biologie, **15**; Meyer, *ibid.*, **7**; Hultgren and Landergren, Nord. med. Arch., **21**; Schuster, in Voit's "Untersuch. d. Kost," etc., 142; Cramer, Zeitschr. f. physiol. Chem., **6**; Meinert, "Ueber Massennährung," Berlin, 1885; Kellner and Mori, Zeitschr. f. Biologie, **25**.

[2] Abelmann, "Ueber die Ausnützung der Nahrungsstoffe nach Pankreasexstirpation" (Inaug.-Dissert. Dorpat, 1890), cited from Maly's Jahresber., **20**; Sandmeyer, Zeitschr. f. Biologie, **31**; Rosenberg, Pflüger's Arch., **70**; Harley, Journ. of Pathol. and Bacteriol., 1895; Deucher, Correspond. Blatt. f. Schweiz. Aerzte, **28**; Lombroso, Arch. f. exp. Path. u. Pharm., **60**; Niemann, Zeitschr. f. exp. Path. u. Therap., **5**.

tion fluids act substitutingly. In this regard ZUNZ and MAYER [1] found that in dogs (meat digestion) the tying of the pancreatic passages is essentially compensated for by an increased secretion of pepsin and other proteolytic enzymes, and that in this case the demolition of the protein in the stomach goes further than in a normal animal.

The carbohydrates are, it seems, chiefly absorbed as monosaccharides. Dextrose, levulose, and galactose are probably absorbed as such. The two disaccharides, saccharose and maltose, ordinarily undergo an inversion in the intestinal tract and are converted into dextrose and levulose. Lactose is also, at least in certain animals, inverted in the intestine. In other mature animals, on the contrary, if the lactase formation is not excited by milk food, the sugar is not inverted or only to a slight extent (VOIT and LUSK, WEINLAND, PORTIER, RÖHMANN and NAGANO), and it probably is absorbed as such in these animals if it does not undergo fermentation, or, as RÖHMANN and NAGANO [2] assumed, if it is not transformed in the intestinal mucosa in some unknown way. An absorption of non-inverted carbohydrates is not improbable, and according to OTTO and v. MERING the portal blood contains, besides dextrose, a dextrin-like carbohydrate after a carbohydrate diet. MOSCATI [3] believes that when homogeneous starch solutions are injected intravenously or subcutaneously the starch is taken up by the organs, namely the spleen, liver and lungs, and is utilized as the starch is changed into glycogen. A part of the carbohydrates is destroyed by fermentation in the intestine, with the formation of lactic and acetic acids and other bodies.

The different varieties of sugars are absorbed with varying degrees of rapidity, but as a general thing absorption occurs very quickly. This absorption takes place more quickly in the upper part of the intestine than in the lower part (RÖHMANN, LANNOIS and LÉPINE, RÖHMANN and NAGANO [4]). It is generally admitted that the simpler sugars are more quickly absorbed than the disaccharides, while the reports as to the absorption of the disaccharides differ somewhat (HÉDON, ALBERTONI, WAYMOUTH REID, RÖHMANN and NAGANO). There seems to be no doubt that lactose is absorbed more slowly than the two other disaccharides. According to the extensive experiments of RÖHMANN and NAGANO, saccharose is absorbed more quickly than maltose. NAGANO [5] contends that the pentoses are absorbed more slowly than hexoses.

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[1] Mem. de l'acad. roy. de médic. de Belg., 18.

[2] Voit and Lusk, Zeitschr. f. Biologie, 28; Röhmann and Nagano, Pflüger's Arch., 95, which contains the references to the literature.

[3] Otto, see Maly's Jahresber., 17; v. Mering, Arch. f. (Anat. u.) Physiol., 1877; Moscati, Zeitschr. f. physiol. Chem., 50.

[4] Lannois and Lépine, Arch. de physiol. (3), 1; Röhmann, Pflüger's Arch., 41; see also footnote 2.

[5] In regard to the literature on the absorption of sugars, see footnote 2.

On the introduction of starch even in very considerable quantities into the intestinal tract no dextrose passes into the urine, a condition which probably depends in this case upon the absorption and assimilation and the slow saccharification taking place simultaneously. If, on the contrary, large quantities of sugar are introduced at one time, then an elimination of sugar by the urine takes place, and this elimination of sugar is called *alimentary glycosuria*. In these cases the assimilation of the sugar and the absorption do not take place together.

That quantity of sugar to which we must raise the ingested substance in order to produce an alimentary glycosuria gives, according to HOFMEISTER,[1] the *assimilation limit* for that same sugar. This limit is different for various kinds of sugar; and it also varies for the same sugar not only in different animals, but also in different members of the same species, as also in the same individual under varying circumstances. In general it can be said that in regard to the ordinary varieties of sugar, such as dextrose, levulose, galactose, saccharose, maltose, and lactose, the assimilation limit is highest for dextrose and lowest for lactose. It must be admitted that with an overabundant quantity of sugars in the intestinal tract the disaccharides do not have sufficient time for their complete inversion, and this has been directly shown by RÖHMANN and NAGANO. It is, therefore, not remarkable that disaccharides, as well, have been found in the urine in cases of alimentary glycosuria.[2]

The investigations of LUDWIG and v. MERING and others have explained how the sugars enter into the blood-stream, namely, that they as well as other bodies soluble in water do not ordinarily pass over into the chylous vessels in measureable quantities, but are chiefly taken up by the blood in the capillaries of the villi and in this way pass into the mass of the blood. These investigations have been confirmed by observations of I. MUNK and ROSENSTEIN [3] on human beings.

The reason why the sugars and other soluble bodies do not pass over into the chylous vessels in appreciable quantity is, according to HEIDENHAIN,[4] to be found in the anatomical conditions, in the arrangement of the capillaries close under the layer of epithelium. Ordinarily these capillaries find the necessary time for the removal of the water and the solids dissolved in it. But when a large quantity of liquid, such as a sugar solution, is introduced into the intestine at once, this is not possible,

[1] Arch. f. exp. Path. u. Pharm., **25** and **26**.

[2] For the literature in regard to the passage of various kinds of sugars into the urine, see C. Voit, Ueber die Glykogenbildung, Zeitschr. f. Biologie, **28**, and F. Voit, footnote 2, p. 375. See also Blumenthal, Zur Lehre von der Assimilationsgrenze der Zuckerarten, Inaug.-Dissert. 1903, Strassburg and Brasch, Zeitschr. f. Biol., **50**.

[3] v. Mering, Arch. f. (Anat. u.) Physiol., 1877; Munk and Rosenstein, Virchow's Arch., **123**.

[4] Pflüger's Arch., **43**, Suppl.

and in these cases a part of the dissolved bodies passes into the chylous vessels and the thoracic duct (GINSBERG and RÖHMANN [1]).

The passage of sugar into the urine when at one time large quantities of sugar are taken and the assimilation limit is exceeded, can be best explained by the assumption that a part of the sugar escaped the liver and passed into the large circulation, or that the liver did not have time to retain the sugar and transform it into glycogen. According to the observations of FILIPPI [2] upon dogs with ECK fistula, it seems as if the rôle of the liver in these cases is too highly estimated. An animal with ECK fistula could take an unlimited quantity of starch without glycosuria occurring. The assimilation limit was in these cases somewhat lower, but qualitatively they behave like normal animals and with increasing sugar supply they could also retain increasing quantities of sugar.

The introduction of larger quantities of sugar into the intestine at one time can readily cause a disturbance with diarrheal evacuations of the intestine. If the carbohydrate is introduced in the form of starch, then very large quantities may be absorbed without causing any disturbance, and the absorption may be very complete. RUBNER found the following: On partaking 508–670 grams of carbohydrates, as wheat bread, per day the part not absorbed amounted to only 0.8–2.6 per cent. For peas, where 357-588 grams were eaten, the loss was 3.6–7 per cent, and for potatoes (718 grams) 7.6 per cent. CONSTANTINIDI found on partaking 367–380 grams of carbohydrates, chiefly as potatoes, a loss of only 0.4–0.7 per cent. In the experiments of RUBNER, as also of HULTGREN and LANDERGREN,[3] with rye bread the utilization of carbohydrates was less complete, and the loss in a few cases rose even to 10.4–10.9 per cent. It at least follows from the experiments made thus far that man can absorb more than 500 grams of carbohydrates per diem without difficulty.

We generally consider the pancreas as the most important organ in the digestion and absorption of amylaceous bodies, and it is a question how these bodies are absorbed after the extirpation of the pancreas. As on the absorption of proteins, so also on the absorption of starch, the observations have given variable results. In certain cases the absorption was not impaired, while in others it was, on the contrary, rather diminished, and with dogs devoid of pancreas it has been found that the absorption was decreased to 50 per cent of the starch partaken (ROSENBERG, CAVAZZANI [4]).

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[1] Ginsberg, Pflüger's Arch., **44**; Röhmann, *ibid.*, **41**.

[2] Zeitschr. f. Biol., **49** and **50**.

[3] Rubner, Zeitschr. f. Biologie, **15** and **19**; Constantinidi, *ibid.*, **23**; Hultgren and Landergren, l. c.

[4] Cavazzani, Centralbl. f. Physiol., **7**. See footnote 2, p. 508; also Lombroso, Hofmeister's Beiträge, 8.

Emulsification used to be considered as of the greatest importance in the absorption of fats, and this emulsion occurs in the chyle on the introduction into the intestine of not only neutral fats, but also of fatty acids. The fatty acids do not exist as such in the emulsified fat of the chyle. The investigations of I. MUNK, later confirmed by others, have shown that the fatty acids undergo in great part a synthesis into neutral fats in the walls of the intestine, and are carried as such by the stream of chyle into the blood. This synthesis seems to take place in the mucous membrane (MOORE and others [1]).

The assumption that the fat is absorbed chiefly as an emulsion is partly based on the abundance of emulsified fat in the chyle after feeding with fat, and partly on the fact that a fat emulsion is often found in the intestine after such food. As an abundant cleavage of neutral fats occurs in the intestinal canal, and also as the fatty acids do not occur in the chyle as such, but as emulsified fat after a synthesis with glycerin into neutral fats, it is to be doubted whether the emulsified fat of the chyle originates from an absorption of emulsified fat in the intestine or from a subsequent emulsification of neutral fats formed synthetically. This doubt has greater warrant in the observation of FRANK [2] that the fatty-acid ethyl ester is extensively taken up from the intestine, not as such, but as split-off fatty acids from which then the neutral emulsified fats of the chyle are formed.

The assumption of an absorption of fats as an emulsion is inconsistent with the fact that an emulsion produced by means of soaps is not permanent in an acid liquid; hence we cannot consider as possible the presence of an emulsion in the intestine so long as it is acid. This difficulty is not too serious, as the reaction is often only due to carbonic acid and bicarbonates, and besides as found by KÜHNE and recently shown by MOORE and KRUMBHOLZ,[3] the proteins have a preserving action upon fat emulsions.

The earlier opinions as to fat absorption were that fat was absorbed as soaps, soluble in water, as well as finely emulsified fat, and this last form was considered as of the greatest importance. This view has recently undergone essential modifications, due to the work of MOORE and ROCKWOOD, and especially to the extensive work of PFLÜGER.[4]

[1] Munk, Virchow's Arch., 80. See also v. Walther, Arch. f. (Anat. u.) Physiol., 1890; Minkowski, Arch. f. exp. Path. u. Pharm., 21; Frank, Zeitschr. f. Biologie, 36; Moore, see Biochem. Centralbl., 1, 741; Frank and Ritter, Zeitschr. f. Biologie, 47.

[2] Zeitschr. f. Biologie, 36.

[3] Kühne, Lehr. der physiol. Chem., 122; Moore and Krumbholz, Journ. of Physiol., 22.

[4] In regard to the recent literature on fat absorption, see the works of Pflüger, Pflüger's Arch., 80, 81, 82, 85, 88, 89, and 90, where the work of other investigators is cited and discussed.

Moore and Rockwood have shown the great solvent action of the bile for fatty acids, and on continuing these investigations further, Moore and Parker have found that the bile increases the solubility of soaps in water and can prevent their gelatinization, a fact which is of greater importance for the absorption of fats than the solubility of the fatty acids in bile. The quantity of lecithin in the bile is of great importance for the solubility therein of the fatty acids as well as the soaps. According to the above-mentioned investigators, the absorption of fat from the intestine is essentially dependent upon the solubility of the soaps and free fatty acids in the bile. The neutral fats are split and the free fatty acids are in part absorbed, dissolved as such by the bile, and in part combined with alkalies, forming soaps. Neutral fats are regenerated from the fatty acids, and the alkali set free from the soaps is secreted again into the intestine and used for the re-formation of soaps.

The importance of the bile, the soaps, and the alkali carbonates has been closely studied, chiefly in the very thorough investigations of Pflüger. He has quantitatively determined the solvent power of the above-mentioned bodies—each alone as well as different mixtures of these—for the various fatty acids, and has closely studied the mode of action of the bile. From his investigations he has arrived at the conclusion that no unsplit fat is absorbed, that all fats, before their absorption, must first be split into glycerin and fatty acids, and that the bile, on account of its solvent power for soaps and fatty acids, is sufficient for the absorption of large quantities of fat eaten. The object of the formation of an emulsion is, according to this view, that the fat in this condition forms such a large surface for the action of the steapsin or the fat-splitting agents.

The possibility that all the fat must be first split and that no unsplit fat is absorbed is, according to these researches, not to be denied. It is the opinion of the author that it is still too early to give a positive verdict as to how these conditions in the intestine are brought about and the conclusion must be left for further investigation.

The next question is whether all the fat or the greater part of it passes into the blood through the lymphatics and the thoracic duct. According to the researches of Walther and Frank[1] on dogs, it seems that only a small part of the fats, or at least of the fatty acids fed, pass into the chylous vessels; but these observations can hardly be applied to the absorption of neutral fats, or to the absorption in man under normal circumstances. Munk and Rosenstein,[2] in their investigations on a girl with a lymph fistula, found 60 per cent of the

[1] Walther, Arch. f. (Anat. u.) Physiol., 1890; Frank, *ibid.*, 1892.

[2] Virchow's Arch., **123**.

fat ingested in the chyle, and of the total quantity of fat in the chyle only 4–5 per cent existed as soaps. On feeding with a foreign fatty acid, such as erucic acid, they found 37 per cent of the introduced body as neutral fat in the chyle. Not all the fat introduced is found in the chyle, and there is always a not inconsiderable part of the absorbed fat whose fate we are not able to follow.

The completeness with which fats are absorbed depends, under normal conditions, essentially upon the kind of fat. In this regard it is known, especially from the investigations of MUNK and ARNSCHINK,[1] that the varieties of fat with high melting-points, such as mutton-tallow, and especially stearin, are not so completely absorbed as the fats with low melting-points, such as hog- and goose-fat, olive-oil, etc. The kind of fat also has an influence on the rapidity of absorption, as MUNK and ROSENSTEIN found that solid mutton-fat was absorbed more slowly than fluid lipanin. The extent of absorption in the intestinal tract is, under physiological conditions, very considerable. In the case of a dog investigated by VOIT it was found that out of 350 grams of fat (butter) partaken, 346 grams were absorbed from the intestinal canal, and according to the investigations of RUBNER[2] the human intestine can absorb over 300 grams of fat per diem. The fats are, according to RUBNER, much more completely absorbed when free, in the form of butter or lard, than when inclosed in cell-membranes, as in bacon.

CLAUDE BERNARD showed long ago with experiments on rabbits in which the ductus choledochus was made to open into the small intestine above the pancreatic duct, that after food rich in fats the chylous vessels of the intestine above the pancreas passages were transparent, while below they were milk-white, and also that the bile alone cannot produce an absorption of the emulsified fat without the pancreatic juice. DASTRE[3] has performed the reverse experiment on dogs. He tied the ductus choledochus and adjusted a biliary fistula so that the bile flowed into the intestine below the mouth of the pancreatic passages. On killing the animal after a meal rich in fat the chylous vessels were first found milk-white below the discharge of the biliary fistula. From this DASTRE draws the conclusion that a combined action of the bile and pancreatic juice is important in the absorption of fats—a conclusion which stands in accord with the experience of many others.

Through numerous observations of many investigators, such as BIDDER and SCHMIDT, VOIT, RÖHMANN, FR. MÜLLER, I. MUNK,[4] and

[1] Munk, Virchow's Arch., **80** and **95**; Arnschink, Zeitschr. f. Biologie, **26**.

[2] Voit, Zeitschr. f. Biologie, **9**; Rubner, *ibid.*, **15**.

[3] Arch. de Physiol. (5), **2**.

[4] F. Müller, Sitzungsber. der phys.-med. Gesellsch. zu Würzburg, 1885; I. Munk, Virchow's Arch., **122**. See also footnotes 4, p. 495 and 1, p. 496.

others, it has been shown that the exclusion of the bile from the intestinal tract diminishes the absorption of fat to such an extent that only one-seventh to about one-half of the quantity of fat ordinarily absorbed undergoes absorption. In icterus with entire exclusion of the bile, a considerable decrease in the absorption of fat is noticed. As under normal conditions, so also in the absence of bile in the intestine, the lower melting parts of the fat are more completely absorbed than those which have a high melting-point. I. MUNK found in his experiments on dogs with lard and mutton-tallow that the absorption of the high-melting tallow was reduced twice as much as the lard on the exclusion of the bile from the intestine.

We also learn from the investigations of RÖHMANN and I. MUNK that in the absence of bile the relation between fatty acids and neutral fats is changed, namely, about 80–90 per cent of the fat existing in the feces consists of fatty acid, while under normal conditions the feces contain 1 part neutral fat to about 2–2½ parts free fatty acids. It is not possible to state how this increased quantity of fatty acids in the fat of the feces is produced upon the exclusion of the bile from the intestine.

There is no doubt that the bile is of great importance in the absorption of fats. Still there is also no doubt that rather considerable quantities of fat may be absorbed from the intestine in the absence of bile. What relation does the pancreatic juice bear to this fact?

Upon this point a rather large number of observations on animals have been made by ABELMANN and MINKOWSKI, SANDMEYER, HARLEY, ROSENBERG, HÉDON and VILLE, and also on man by FR. MÜLLER and DEUCHER.[1] In all of these investigations a more or less diminished absorption of fat was observed after the extirpation or destruction of the gland, or the exclusion of the juice from the intestine. The results are very diverse as to the extent of this diminution, as in certain cases no absorption of fat was observed, while, in other cases, a considerable absorption was noted in the same class of animal (dog) and even in the same animal. According to MINKOWSKI and ABELMANN, after the total extirpation of the pancreas the fat of the food introduced is not absorbed at all, with the exception of milk, of which 28–53 per cent of the fat is absorbed. Other investigators have obtained other results, and HARLEY has observed a case where in a dog an absorption of only 4 per cent of the milk fat, or, on the complete exclusion of intestinal bacteria, even

[1] Müller, "Unters. über den Icterus," Zeitschr. f. klin. Med., **12**; Hédon and Ville, Arch. de Physiol. (5), **9**; Harley, Journ. of Physiol., **18**, Journ. of Pathol. and Bacteriol., 1895, and Proceed. Roy. Soc., **61**. In regard to the other authors see footnote 2, p. 508.

no absorption, took place. The conditions may vary in the different cases, and the behavior is not the same in different varieties of animals.

As shown by LOMBROSO, there exists an essential difference between the action of the extirpation of the gland or a prevented flow of the secretion into the intestine. In the last case, as the experiments reported by NIEMANN show, no essential disturbance of the absorption takes place, while the total extirpation of the gland is followed by a marked disturbance (LOMBROSO [1]). This investigator is also of the opinion that the pancreas, independent of the external secretion in any way (by endocrinic bodies), influences the absorption of the foodstuffs and the activity of the pancreas enzymes in the intestine. In order to judge this view it would be of the greatest interest to know how the exclusion of the pancreatic juice from the intestine acts upon the other factors of the digestion, such as upon the formation of the secretions and their activity. As to this we know at present very little, but the work of ZUNZ and MAYER (see page 509), indicates that such a reverse action is possible. Under these circumstances it is not possible to give LOMBROSO's views too great a prominence.

LOMBROSO has also found that after the extirpation of the pancreas in the dog sometimes more fat is eliminated than was contained in the food; that this eliminated fat, which depends upon a fat secretion in the intestinal canal, has a different composition from the introduced fat, and that in these cases an absorption of fat also takes place. That some fat can be absorbed in animals even in the absence of the bile as well as pancreatic juice has been shown by the investigations of HÉDON and VILLE and CUNNINGHAM.[2]

The reason for the fact that the fat absorption is diminished in the absence of bile from the intestine must be sought for in the above-mentioned rôle of this fluid. It is more difficult to state why the absence of pancreatic juice causes a reduction in the absorption of fat. The most natural view is that the neutral fats are here less completely split, but this does not seem to be the case, because the non-absorbed fat of the feces consists, on the exclusion of bile and pancreatic juice (MINKOWSKI and ABELMANN, HARLEY, HÉDON and VILLE, DEUCHER), chiefly of free fatty acids. A still unknown change caused by gastric or intestinal lipase or by micro-organisms may produce a cleavage of the fat in these cases. The imperfect fat absorption after the extirpation of the pancreas can possibly be explained by the removal of a considerable part of the alkalies necessary for the formation of the emulsion and for the solution

[1] Lombroso, see Bioch. Centralbl., **3**, 67 and 566, and **4**, 738; also Compt. rend. soc. biol., **57**; Hofmeister's Beiträge, 8, **11**; Pflüger's Arch., **112**; and Arch. f. exp. Path. u. Pharm., **56** and **60**; Niemann, l. c.

[2] Hédon and Ville, l. c.; Cunningham, Journ. of Physiol., **23**.

of the fatty acids, but as SANDMEYER found in dogs deprived of their pancreas, that the fat absorption was raised by giving chopped pancreas with the fat, this can hardly be a sufficient explanation. The reason for this is perhaps that after the extirpation of the pancreas the splitting of the fat is chiefly brought about by bacteria in those parts of the intestinal canal where the conditions for absorption are not favorable.

The soluble salts are also absorbed with the water. The proteins, which can dissolve a considerable quantity of salts, such as earthy phosphates which are otherwise insoluble in alkaline water, are of great importance in the absorption of such salts.

The enzymes may also be absorbed like other soluble constituents of the digestive secretions, as is demonstrated by the passage of pepsin into urine. The occurrence of urobilin in urine attests the absorption of the bile-constituents under physiological conditions despite the fact that the occurrence of very small traces of bile-acids in the urine is disputed. The absorption of bile-acids by the intestine seems to be positively proven by other observations. TAPPEINER [1] introduced a solution of bile-salts of a known concentration into an intestinal knot and after a time investigated the contents. He found that in the jejunum and the ileum, but not in the duodenum, an absorption of bile-acids took place, and further that of the two bile acids only the glycocholic acid was absorbed in the jejunum. Further, SCHIFF long ago expressed the opinion that bile undergoes an intermediate circulation, in such wise that it is absorbed from the intestine, then carried to the liver by the blood, and lastly eliminated from the blood by this organ. Although this view has met with some opposition, still its correctness seems to be established by the researches of various investigators, and more recently by PREVOST and BINET, and specially by STADELMANN and his pupils.[2] After the introduction of foreign bile into the intestine of an animal the foreign bile-acids appear again in the secreted bile.

How does the removal of large portions of the various parts of the intestine affect absorption? HARLEY [3] has been able to perform a partial extirpation of the large intestine and in another instance a complete extirpation. This last condition increased the feces considerably, especially because of the large increase in the water (fivefold). Fats and carbohydrates were absorbed just as completely as in the normal. The absorption of the proteins, on the contrary, was reduced to only 84 per cent as compared to 93–98 per cent in normal dogs. After extirpation the feces sometimes did not contain any urobilin, or only traces thereof, while bile-pigments existed in large amounts.

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[1] Wien. Sitzungsber., **77**.

[2] Schiff, Pflüger's Arch., **3**; Prevost and Binet, Compt. rend., **106**; Stadelmann, see footnote 1, p. 394.

[3] Proceed. Roy. Soc., **64**.

Erlanger and Hewlett[1] found that dogs from which 70–83 per cent of the total length of the jejunum and ileum had been removed, could be kept alive, like other animals, if only the food was not too rich in fat. When the food contained large amounts of fat then 25 per cent was evacuated by the feces as compared to 4–5 per cent in the normal animal. Under these same conditions the amount of nitrogen in the feces was increased to twice the normal amount.

After the exclusion of the colon in rabbits, Bergmann and Hultgren[2] could find no definite action upon the availability of the cellulose nor could any diminution in the utility of the other constituents of the food be observed. Zuntz and Ustjanzew[3] also found that the removal of the cæcum had no influence on the utilization of nitrogen; but in respect of other factors they arrived at different results. They found, namely, that the cæcum of the rodent is of great importance for the digestion of crude fibre and the pentosans. On feeding hay and wheat to rabbits after the removal of the cæcum, the digestion coefficient for crude fibre fell from 42.8 to 23.4–18.7 per cent, and for pentosans from 50 to 40–28.7 per cent.

The question as to the forces which are active in the intestine during absorption has not been answered. It is certain that thus far the laws of diffusion and osmosis alone are not sufficient to explain absorption, although some claim that it is. With all these facts in view, and as it is not within the scope of this book to enter more in detail upon the numerous investigations of this subject, we must refer to larger works[4] and to text-books on physiology for further information.

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[1] Amer. Journ. of Physiol., **6**.

[2] Skand. Arch. f. Physiol., **14**.

[3] Verhandl. d. physiol. Gesellsch. zu Berlin, 1904–1905.

[4] See Höber, Physikalische Chemie der Zelle, Leipzig, 1906, and I. Munk, Ergebnisse der Physiologie, I, Abt. 1; Hamburger, Osmotischer Druck und Ionenlehre, Bd. 2, Weisbaden, 1904.

CHAPTER X.

TISSUES OF THE CONNECTIVE SUBSTANCE.

I. THE CONNECTIVE TISSUES.

THE form-elements of the typical connective tissues are cells of various kinds, of a not very well-known chemical composition, and gelatin-yielding fibrils, which, like the cells, are imbedded in an interstitial or intercellular substance. The fibrils consist of *collagen*, the interstitial substance contains chiefly *mucoid* (*tendon-mucoid*), besides *serglobulin* and *seralbumin*, which occur in the parenchymatous fluid (LOEBISCH [1]).

The connective tissue also often contains fibres or formations consisting of *elastin*, sometimes in such great quantities that the connective tissue is transformed into elastic tissue. A third variety of fibres, the reticular fibres, also occurs, and according to SIEGFRIED these consist of *reticulin*.

If finely divided tendons are extracted in cold water or NaCl solutions, the protein bodies soluble in the nutritive fluid in addition to a little mucoid are dissolved. If the residue is extracted with half-saturated lime-water, then the mucoid is dissolved and may be precipitated from the filtered extract by adding an excess of acetic acid. The extracted residue contains the fibrils of the connective tissue together with the cells and the elastic substance.

The so-called tendon mucin is not true mucin, but a mucoid, which, as first shown by LEVENE and then by CUTTER and GIES, contains a part of its sulphur as an acid related to chondroitin-sulphuric acid. These mucoids, which according to CUTTER and GIES are mixtures of several glycoproteins, contain 2.2–2.33 per cent sulphur, as shown by the analyses of CHITTENDEN and GIES, as well as those of CUTTER and GIES. The quantity of sulphur split off as sulphuric acid was 1.33–1.62 per cent (CUTTER and GIES [2]).

The fibrils of the connective tissue are elastic and swell slightly in water, somewhat more in dilute alkalies or in acetic acid. On the other hand, they shrink by the action of certain metallic salts, such as ferrous

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[1] Zeitschr. f. physiol. Chem., **10**.

[2] Levene, *ibid.*, **31** and **39**; Cutter and Gies, Amer. Journ. of Physiol., **6**; Chittenden and Gies, Journ. of Exp. Med., **1**.

sulphate or mercuric chloride, and tannic acid, which form insoluble compounds with the collagen. Among these compounds, which prevent putrefaction of the collagen, that with tannic acid has been found of the greatest technical importance in the preparation of leather. In regard to the collagens, gelatins, elastins, and reticulins, see pages 115 to 120.

The tissues described under the names *mucous* or *gelatinous tissues* are characterized more by their physical than by their chemical properties, and have been but little studied. This much, however, is known, that the mucous or gelatinous tissues contain, at least in certain cases, as in the Acalephæ, no mucin.

The umbilical cord is the most accessible material for the investigation of the chemical constituents of the gelatinous tissues. The mucin occurring therein has been described on page 166. C. Th. Mörner[1] has found a *mucoid* in the vitreous humor which contains 12.27 per cent nitrogen and 1.19 per cent sulphur.

Young connective tissue is richer in mucoid than old. Halliburton[2] found an average of 7.66 p. m. mucoid in the skin of very young children and only 3.85 p. m. in the skin of adults. In so-called myxœdema, in which a re-formation of the connective tissue of the skin takes place, the quantity of mucoid is also increased.

The connective tissue and also the elastic tissue are richer in water and poorer in solids in young animals as compared with full-grown animals. This may be seen from the following analyses of the Achilles tendon (Buerger and Gies) and of the ligamentum nuchæ (Vandegrift and Gies[3]):

	Achilles tendon.		Ligament.	
	Calf.	Ox.	Calf.	Ox.
Water	675.1 p. m.	628.7 p. m.	651.0 p. m.	575.7 p. m.
Solids	324.9 "	371.3 "	394.0 "	424.3 "
Organic bodies	318.4 "	366.6 "	342.4 "	419.6 "
Inorganic bodies	6.1 "	4.7 "	6.6 "	4.7 "
Fat	.....	10.4 "	.....	11.2 "
Proteid	.....	2.2 "	.....	6.16 "
Mucoid	.....	12.83 "	.....	5.25 "
Elastin	.....	16.33 "	.....	316.70 "
Collagen	.....	315.88 "	.....	72.30 "
Extractives, etc	.....	8.96 "	.....	7.99 "

In regard to the mineral bodies it must be remarked that according to the determinations of H. Schulz the connective tissue is rich in silicic acid. The greatest amount was found by him, in the crystalline lens

[1] Zeitschr. f. physiol. Chem., 18, 250.

[2] Mucin in Myxœdema: Further Analyses. King's College Collected Papers No. 1, 1893.

[3] Buerger and Gies, Amer. Journ. of Physiol., 6; Vandegrift and Gies, *ibid.*, 5.

of the ox, namely, 0.5814 gram per kilo of dried substance. In man he found 0.0637 gram in the tendons, 0.1064 gram in the fascia, and 0.244 gram in Wharton's jelly for every kilo of dried substance. The quantity of silicic acid is higher in the young than in the old; in man it is highest in the embryonic connective tissue of the umbilical cord. In the last-named substance SCHULZ also found 0.403 gram Fe_2O_3, 0.693 gram MgO, 3.297 grams CaO, and 3.794 grams P_2O_5 for every kilo of dried substance. The report of SCHULZ on the quantity of silicic acid does not correspond with the recent investigations of FRAUENBERGER[1] who found, in Wharton's jelly, about one-twentieth the quantity of silicic acid that SCHULZ gives.

II. CARTILAGE.

Cartilaginous tissues consist of cells and an original hyaline matrix, which, however, may become changed in such wise that there appears in it a network of elastic fibres or connective-tissue fibrils.

Those cells that offer great resistance to the action of alkalies and acids have not been carefully studied. According to earlier opinions the matrix was considered as consisting of a body analogous to collagen, so-called *chondrigen*. The recent investigations of MOROCHOWETZ and others, but especially those of C. MÖRNER,[2] have shown that the matrix of the cartilage consists of a mixture of collagen with other bodies.

The tracheal, thyroideal, cricoidal, and arytenoidal cartilages of full-grown cattle contain, according to MÖRNER, four constituents in the matrix, namely, *chondromucoid, chondroitin-sulphuric acid, collagen,* and the *albumoid.*

Chondromucoid. This body, according to C. MÖRNER, has the composition C 47.30, H 6.42, N 12.58, S 2.42, O 31.28 per cent. Sulphur is in part loosely combined and may be split off by the action of alkalies, and a part separates as sulphuric acid when boiled with hydrochloric acid. Chondromucoid is decomposed by dilute alkalies and yields alkali albuminate, peptone substances, chondroitin-sulphuric acid, alkali sulphides, and some alkali sulphates. On boiling with acids it yields acid albuminate, peptone substances, chondroitin-sulphuric acid, and on account of the further decomposition of this last body, sulphuric acid and a reducing substance are formed.

Chondromucoid is a white, amorphous, acid-reacting powder which is insoluble in water, but dissolves easily on the addition of a little alkali. This solution is precipitated by acetic acid in great excess and by small

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[1] Schulz, Pflüger's Arch., 84 and 89; Frauenberger, Zeitschr. f. physiol. Chem., 57.

[2] Morochowetz, Verhandl. d. naturh. med. Vereins zu Heidelberg, 1, Heft 5; Mörner, Skand. Arch. f. Physiol., 1.

quantities of mineral acids. The precipitation may be retarded by neutral salts or by chondroitin-sulphuric acid. The solution containing NaCl and acidified with HCl is not precipitated by potassium ferrocyanide. Precipitants for chondromucoid are alum, ferric chloride, sugar of lead, or basic lead acetate. Chondromucoid is not precipitated by tannic acid, and it may by its presence prevent the precipitation of gelatin by this acid. It gives the usual color reactions for proteins, namely, with nitric acid, with copper sulphate and alkali, with MILLON'S and ADAMKIEWICZ'S reagents.

Chondroitin-sulphuric Acid, CHONDROITIC ACID. This acid, which was first prepared pure, from cartilage, by C. MÖRNER and identified by him as an ethereal sulphuric acid, occurs, according to MÖRNER, in all varieties of cartilage and also in the tunica intima of the aorta and as traces in the bone substance. K. MÖRNER [1] has also found it in the ox-kidney and in human urine as a regular constituent. Its occurrence in amyloid as mentioned on page 168 has been disputed by HANSSEN. In the opinion of LEVENE,[2] the glucothionic acid which is prepared from tendon mucoid and which gives the orcin reaction for glucuronic acid, and yields furfurol on distillation with hydrochloric acid, is not identical with the chondroitin-sulphuric acid, but is probably related thereto.

Chondroitin-sulphuric acid has the formula $C_{18}H_{27}NSO_{17}$, according to SCHMIEDEBERG.[3] As primary products this acid yields, on cleavage, sulphuric acid and a nitrogenous substance, *chondroitin*, according to the following equation:

$$C_{18}H_{27}NSO_{17}+H_2O=H_2SO_4+C_{18}H_{27}NO_{14}.$$

Chondroitin, which is similar to gum arabic, and which is a monobasic acid, yields acetic acid and a new nitrogenous substance, *chondrosin*, as cleavage products, on decomposition with dilute mineral acids:

$$C_{18}H_{27}NO_{14}+3H_2O=3C_2H_4O_2+C_{12}H_{21}NO_{11}.$$

Chondrosin, which is also a gummy substance soluble in water, is a monobasic acid and reduces copper oxide in alkaline solutions even more strongly than dextrose. It is dextrogyrate, and represents the reducing substance obtained by previous investigators in an impure form on boiling cartilage with an acid. The products obtained on decomposing chondrosin with barium hydroxide tend to show, according to SCHMIEDEBERG, that chondrosin contains the atomic groups of glucuronic

[1] C. Mörner, l. c., and Zeitschr. f. physiol. Chem., **20** and **23**; K. Mörner, Skand. Arch. f. Physiol., **6**.

[2] Zeitschr. f. physiol. Chem., **39**.

[3] Arch. f. exp. Path. u. Pharm., **28**.

acid and glucosamine. This assumption does not seem to have sufficient foundation. According to ORGLER and NEUBERG, chondrosin does not give the orcin test nor does it yield furfurol. They claim that on cleavage with baryta it yields, besides a carbohydrate complex which has not been studied, an oxyamino-acid having the formula $C_6H_{13}O_6N$ and also a hexosamine acid or tetraoxyaminocaproic acid. In opposition to this S. FRÄNKEL [1] has found that the chondrosin gives the orcin as well as the phloroglucin test with hydrochloric acid, and he has prepared an acid with the formula $C_6H_{11}NO_6$, which he calls *aminoglucuronic acid*, which gives the above tests and also reduces.

Chondroitin-sulphuric acid appears as a white amorphous powder which dissolves very easily in water, forming an acid solution and, when sufficiently concentrated, a sticky liquid similar to a solution of gum arabic. Nearly all of its salts are soluble in water. The neutralized solution is precipitated by stannous chloride, basic lead acetate, neutral ferric chloride, and by alcohol in the presence of a little neutral salt. The solution, on the other hand, is not precipitated by acetic acid, tannic acid, potassium ferrocyanide and acid, sugar of lead, mercuric chloride, or silver nitrate. Acidified solutions of alkali chondroitin-sulphates cause a precipitation when added to solutions of gelatin or proteid.

The preparation of chondromucoid and its separation from chondroitin-sulphuric acid can be accomplished after the method of C. MÖRNER, but for details we refer to the original work.

The pre-existing chondroitin-sulphuric acid, or that formed by the decomposition of chondromucoid, is obtained by lixiviating the cartilage with a 5-per cent caustic-alkali solution. The alkali albuminate formed by the decomposition of the chondromucoid can be removed from the solution by neutralization, then the peptone precipitated by tannic acid, the excess of this acid removed with sugar of lead, and the lead removed from the filtrate by H_2S. If further purification is necessary, the acid is precipitated with alcohol, the precipitate dissolved in water, this solution dialyzed and precipitated again with alcohol,—this solution in water and precipitation with alcohol being repeated a few times,—and lastly the acid is treated with alcohol and ether.

SCHMIEDEBERG prepared the acid from the septum narium of the pig according to the following method: The finely divided cartilage is first exposed to artificial peptic digestion, then carefully washed with water and the insoluble residue treated with 2–3 per cent hydrochloric acid. This cloudy liquid containing hydrochloric acid is precipitated with alcohol (about one-quarter vol.) and the clear filtrate treated with absolute alcohol and some ether. The precipitate, consisting chiefly of a combination or a mixture of chondroitin-sulphuric acid and gelatin peptone (peptochondrin), is first washed with alcohol and then with water.

[1] Orgler and Neuberg, Zeitschr. f. physiol. Chem., **37**; Fränkel, Annal. d. Chem. u. Pharm., **351**.

It is then dissolved in alkaline water and the basic alkali compound precipitated from this solution by the addition of alcohol, whereby the gelatin-peptone alkali remains in solution. The precipitate is purified by repeated solution in alkaline water and precipitated by alcohol. To obtain chondroitin-sulphuric acid entirely free from chondroitin it is more advantageous to prepare the potassium-copper compound of the acid, from the alkaline solution, by the alternate addition of copper acetate and caustic potash and precipitation with alcohol. The reader is referred to the original article for more details and also for ODDI's method.

The *collagen* of the cartilage gives, according to C. MÖRNER, a gelatin which contains only 16.4 per cent N and which can hardly be considered identical with ordinary gelatin.

In the above-mentioned cartilages of full-grown animals the chondroitin-sulphuric acid and chondromucoid, perhaps also the collagen, are found surrounding the cells as round balls or lumps. These balls (MÖRNER'S *chondrin-balls*), which give a blue color with methyl-violet, lie in the meshes of a trabecular structure, which is colored when brought in contact with tropæolin.

The *albumoid* is a nitrogenized body which contains loosely combined sulphur. It is soluble with difficulty in acids and alkalies and resembles keratin in many respects, but differs from it by being soluble in gastric juice. In other respects it resembles elastin, but differs from this substance in containing sulphur. This albumoid gives the color reactions of the protein bodies.

Cartilage gelatin and the albumoid may be prepared according to the following method of MÖRNER: First remove the chondromucoid and chondroitin-sulphuric acid by extraction with dilute caustic potash (0.2–0.5 per cent), remove the alkali from the remaining cartilage by water, and then boil with water in a PAPIN'S digester. The collagen passes into solution as gelatin, while the albumoid remains undissolved (contaminated by the cartilage-cells). The gelatin may be purified by precipitating with sodium sulphate, which must be added to saturation in the faintly acidified solution, redissolving the precipitate in water, dialyzing well, and precipitating with alcohol.

In MÖRNER'S experience no albumoid is found in young cartilage, but only the three first-mentioned constituents. Nevertheless the young cartilage contains about the same amounts of nitrogen and mineral substances as the old. The cartilage of the ray (*Raja batis* LIN.), which has been investigated by LÖNNBERG,[1] contains no albumoid and only a little chondromucoid, but a large proportion of chondroitin-sulphuric acid and collagen.

According to PFLÜGER and HÄNDEL,[2] glycogen occurs to a slight

[1] Maly's Jahresber., 19, 325.

[2] Pflüger's in Pflüger Arch., 92; Händel, *ibid.*

extent in all matrices, and of these it is richest in the cartilage. Tendons, ligamentum nuchæ, and cartilage of the ox contained 0.06, 0.07, and 2.17 p. m. glycogen respectively (HÄNDEL).

HOPPE-SEYLER found in fresh human rib-cartilage 676.7 p. m. water, 301.3 p. m. organic and 22 p. m. inorganic substance, and in the cartilage of the knee-joint 735.9 p. m. water, 248.7 p. m. organic and 15.4 p. m. inorganic substance. PICKARDT found 402–574 p. m. water and 72.86 p. m. ash (no iron) in the laryngeal cartilage of oxen. The ash of cartilage contains considerable amounts (even 800 p. m.) of alkali sulphate, which probably does not exist originally as such, but is produced in great part by the incineration of the chondroitin-sulphuric acid and the chondromucoid. The analyses of the ash of cartilage therefore cannot give a correct idea of the quantity of mineral bodies existing in this substance. The cartilage is richest in sodium of all the tissues of the body, and according to BUNGE[1] the amount of Na and Cl is greatest in young animals. In 1000 parts of cartilage dried at 120° C., BUNGE found 91.26 parts Na_2O in the shark, 33.98 in the ox embryo, 32.45 in a fourteen-day-old calf, and 26.4 in a ten-weeks-old calf.

Ochronose is the brown to black coloration of the cartilage which sometimes occurs and which has also been observed in several cases of alcaptonuria (see Chapter XV). The nature of these melanine-like pigments is unknown.

The Cornea. The corneal tissue, which, in a chemical sense, is considered by many investigators to be related to cartilage, contains traces of proteid and a *collagen* as chief constituent, which C. MÖRNER[2] claims contains 16.95 per cent N. According to him it also contains a *mucoid* which has the composition C 50.16, H 6.97, N 12.79, and S 2.07 per cent. On boiling with dilute mineral acid this mucoid yields a reducing substance. The globulins found by other investigators in the cornea are not derived from the matrix, according to MÖRNER, but from the layer of epithelium. MÖRNER believes that DESCEMET'S membrane consists of *membranin* (page 167), which contains 14.77 per cent N and 0.90 per cent S.

In the cornea of oxen HIS[3] found 758.3 p. m. water, 203.8 p. m. gelatin-forming substance, 28.4 p. m. other organic substance, besides 8.1 p. m. soluble and 1.1 p. m. insoluble salts.

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[1] Hoppe-Seyler, cited from Kühne's Lehrbuch d. physiol. Chem., 387; Pickardt, Centralbl. f. Physiol., 6, 735; Bunge, Zeitschr. f. physiol. Chem., 28.

[2] Zeitschr. f. physiol. Chem., 18.

[3] Cited from Gamgee, Physiol. Chem., 1880, 451.

III. BONE.

The bony structure proper, when free from other formations occurring in bones, such as marrow, nerves, and blood-vessels, consists of cells and a matrix.

The *cells* have not been closely studied in regard to their chemical constitution. On boiling with water they yield no gelatin. They contain no keratin, which is not usually present in the bony structure (Herbert Smith [1]).

The *matrix* of the bony structure contains two chief constituents, namely, an organic substance, and the so-called *bone-earths*, lime-salts, inclosed in or combined with it. If bones are treated with dilute hydrochloric acid at the ordinary temperature, the lime-salts are dissolved and the organic substance remains as an elastic mass, preserving the shape of the bone.

The organic matrix consists chiefly of *ossein*, which is generally considered as identical with the collagen of the connective tissue. It also contains, as Hawk and Gies [2] have shown, *mucoid* and *albuminoid*. After the removal of the lime-salts by hydrochloric acid of 2–5 p. m. these experimenters were able to extract the mucoid by one-half saturated lime-water and to precipitate it with 2 p. m. hydrochloric acid. After the removal of the osseomucoid and collagen (by boiling with water) they obtained the albuminoid as an insoluble residue.

The osseomucoid on boiling with hydrochloric acid yielded a reducing substance and sulphuric acid, 1.11 per cent sulphur appearing in this form. The osseomucoid stands close to the chondro- and tendon mucoid in elementary composition, as may be seen from the following analyses:

	C	H	N	S	O	
Osseomucoid.....	47.43	6.63	12.22	2.32	31.40	(Hawk and Gies)
Chondromucoid....	47.30	6.42	12.58	2.42	31.28	(C. Mörner)
Tendon mucoid....	48.76	6.53	11.75	2.33	30.60	(Chittenden and Gies)
Corneal mucoid....	50.16	6.97	12.79	2.07	28.01	(C. Mörner)

The osseoalbuminoid is insoluble in 2 p. m. hydrochloric acid and in 5 p.m. Na_2CO_3, but dissolves in 10 per cent KOH with the formation of albuminates. The composition of chondro- and osseoalbuminoid is as follows:

	C	H	N	S	O	
Osseoalbuminoid.....	50.16	7.03	16.17	1.18	25.46	Hawk and Gies
Chondroalbuminoid ...	50.46	7.05	14.95	1.86	25.68	

The inorganic constituents of the bony structure, the so-called *bone-earths*, which after the complete calcination of the organic substance remain as a white brittle mass, consist chiefly of calcium and phosphoric

[1] Zeitschr. f. Biologie, **19**.

[2] Amer. Journ. of Physiol., **5** and **7**.

acid, but also contain carbonic acid and, in smaller amounts, magnesium, chlorine, and fluorine. Alkali sulphate and iron, which have been found in bone-ash, do not seem to belong exactly to the bony substance, but to the nutritive fluids or to the other constituents of bones. The traces of sulphate occurring in the bone-ash are derived, according to MÖRNER,[1] from the chondroitin-sulphuric acid. According to GABRIEL, potassium and sodium are essential constituents of bone-earth, and this has been substantiated by ARON.[2]

The opinions of investigators differ slightly as to the manner in which the mineral bodies of the bony structure are combined with each other. Chlorine is present in the same form as in apatite ($CaCl_2,3Ca_3P_2O_8$). If we eliminate the magnesium, the chlorine, and the fluorine, the last, GABRIEL claims, occurring only as traces, the remaining mineral bodies form the combination $3(Ca_3P_2O_8)CaCO_3$. In his opinion the simplest expression for the composition of the ash of bones and teeth is $(Ca_3(PO_4)_2+Ca_5HP_3O_{13}+Aq)$, in which 2–3 per cent of the lime is replaced by magnesia, potash, and soda, and 4–6 per cent of the phosphoric acid by carbonic acid, chlorine, and fluorine.

Analyses of bone-earths have shown that the mineral constituents exist in rather constant proportions, which are nearly the same in different animals. As an example of the composition of bone-earth we here give the analyses of ZALESKY.[3] The figures represent parts per thousand:

	Man.	Ox.	Tortoise.	Guinea-pig.
Calcium phosphate, $Ca_3P_2O_8$	838.9	860.9	859.8	873.8
Magnesium phosphate, $Mg_3P_2O_8$	10.4	10.2	13.6	10.5
Calcium combined with CO_2, Fl, and Cl.	76.5	73.6	63.2	70.3
CO_2	57.3	62.0	52.7	.....
Chlorine	1.8	2.0	.....	1.3
Fluorine[4]	2.3	3.0	2.0	.....

Some of the CO_2 is always lost on calcining, so that the bone-ash does not contain the entire CO_2 of the bony substance.

AD. CARNOT[5] found the following composition for the bone-ash of man, ox, and elephant:

	Man.		Ox.	Elephant.
	Femur (body).	Femur (head).	Femur	Femur.
Calcium phosphate	874.5	878.7	857.2	900.3
Magnesium phosphate	15.7	17.5	15.3	19.6
Calcium fluoride	3.5	3.7	4.5	4.7
Calcium chloride	2.3	3.0	3.0	2.0
Calcium carbonate	101.8	92.3	119.6	72.7
Iron oxide	1.0	1.3	1.3	1.5

[1] Zeitschr. f. physiol. Chem., **23**.

[2] Gabriel, *ibid.*, **18**, which also contains the pertinent literature; Aron, Pflüger's Arch., **106**.

[3] Hoppe-Seyler, Med.-chem. Untersuch., p. 19.

[4] The reports as to the quantity of fluorine disagree; see Harms, Zeitschr. f. Biologie, **38**; Jodlbauer, *ibid.*, **41**.

[5] Compt. rend., **114**.

The quantity of organic substance in the bones, calculated from the loss of weight in burning, varies between 300 and 520 p. m. This variation may in part be explained by the difficulty in obtaining the bony substance entirely free from water and partly by the very variable amount of blood-vessels, nerves, marrow, and the like in different bones. The unequal amounts of organic substance found in the compact and in the spongy parts of the same bone, as well as in bones at different periods of development in the same animal, probably depend upon the varying quantities of these above-mentioned tissues. *Dentin*, which is comparatively pure bony structure, contains only 260–280 p. m. organic substance, and HOPPE-SEYLER[1] therefore thinks it probable that perfectly pure bony substance has a constant composition and contains only about 250 p. m. organic substance. The question whether these substances are chemically combined with the bone-earths or only intimately mixed has not been decided.

The nutritive fluids which circulate through the bones have not been isolated and we only know that they contain some protein and some NaCl and alkali sulphate.

Bone Marrow. We differentiate between the red and yellow marrow, to which also belongs the gelatinous marrow poor in fat found in fat atrophy and in old age. The difference between the first two-mentioned kinds of marrow lies, essentially, in the fact that the red marrow contains a greater quantity of erythrocytes besides a higher content of protein and less fat. The fat of the yellow marrow is, according to NERKING,[2] richer in oleic acid and poorer in solid fats than the fat of the red marrow. Besides the fat, lecithin also occurs in the bone-marrow and this varies in amount in different animals and at various ages, as mentioned on page 235. The protein consists of a globulin coagulating at 47–60° C. (FORREST) and a nucleoprotein with 1.6 per cent phosphorus (HALLIBURTON[3]) besides fibrinogen (P. MÜLLER[4]), traces of albumin and proteose. In the extractives are formed lactic acid, inosite, hypoxanthine, cholesterine and bodies of an unknown kind. The quantitative composition of both kinds of marrow varies considerably with the fat content, and the reports of the different investigators are correspondingly discrepant (NERKING, HUTCHINSON and MACLEOD[5]).

The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other tissues,

[1] Physiol. Chem., 102–104.

[2] Bioch. Zeitschr., **10**.

[3] Forrest, Journ. of Physiol., **17**; Halliburton, *ibid.*, **18**.

[4] See footnote 1, p. 245.

[5] Nerking, l. c.; Hutchinson and Macleod, Journ. of Anat. and Physiol., **36**.

such as marrow, blood-vessels, etc., which they contain. The same reason explains, to all appearances, the larger quantity of organic substance in the spongy part of the bones as compared with the more compact parts. SCHRODT [1] has made comparative analyses of different parts of the skeleton of the same animal (dog) and has found an essential difference. The quantity of water in the fresh bones varies between 138 and 443 p. m. The bones of the extremities and the skull contain 138–222, the vertebræ 168–443, and the ribs 324–356 p. m. water. The quantity of fat varies between 13 and 269 p. m. The largest amount of fat, 256–269 p. m., is found in the long tubular bones, while only 13–175 p. m. fat is found in the small short bones. The quantity of organic substance, calculated from fresh bones, was 150–300 p. m., and the quantity of mineral substances 290–563 p. m. Contrary to the general supposition the greatest amount of bone-earths was not found in the femur, but in the first three cervical vertebræ. In birds the tubular bones are richer in mineral substances than the flat bones (DÜRING), and the greatest quantity of mineral bodies has been found in the humerus (HILLER, DÜRING [2]).

We do not possess trustworthy information in regard to the composition of bones at different ages. The analyses by E. VOIT of bones of dogs, and by BRUBACHER of bones of children, apparently indicate that the skeleton becomes poorer in water and richer in ash with increase in age. GRAFFENBERGER [3] has found in rabbits, 6½–7½ years old, that the bones contained only 140–170 p. m. water, while the bones of the full-grown rabbit 2–4 years old contained 200–240 p. m. The bones of old rabbits contain more carbon dioxide and less calcium phosphate.

The composition of bones of animals of different species is but little known. The bones of birds contain, as a rule, somewhat more water than those of mammalia, and the bones of fishes contain the largest quantity of water. The bones of fishes and amphibians contain a greater amount of organic substance. The bones of pachyderms and cetaceans contain a large proportion of calcium carbonate; those of granivorous birds always contain silicic acid. The bone-ash of amphibians and fishes contains sodium sulphate. The bones of fishes seem to contain more soluble salts than the bones of other animals.

A great many experiments have been made to determine the exchange of material in the bones—for instance, with food rich in lime and with food deficient in lime—but the results have always been doubtful or contradictory. The attempts to substitute other alkaline earths or alumina for the lime of the bones have also given conflicting results.[4]

[1] Cited from Maly's Jahresber., 6.

[2] Hiller, cited from Maly's Jahresber., 14; Düring, Zeitschr. f. physiol. Chem., 23.

[3] Voit, Zeitschr. f. Biologie, 16; Brubacher, *ibid.*, 27; Graffenberger in Maly's Jahresber., 21.

[4] See H. Weiske, Zeitschr. f. Biologie, 31, and W. Stoeltzner, Pflüger's Arch., 122, and H. Stoeltzner, Bioch. Zeitschr., 12.

On feeding sufficient calcium and phosphorus in the food ARON [1] found, by strongly reducing the sodium and at the same time giving a large amount of potassium, that the development of the bones was below normal. On the administration of madder the bones of the animal are found to be colored red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism in the bones.

Under pathological conditions, as in rachitis and softening of the bones, an ossein has been found which does not give any typical gelatin on boiling with water. Otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relation between the organic and the inorganic substance. In exostosis and osteosclerosis the quantity of organic substance is generally increased. In rachitis and osteomalacia the quantity of bone-earths is considerably decreased. Attempts have been made to produce rachitis in animals by the use of food deficient in lime. From experiments on fully developed animals opposing results have been obtained. In young, undeveloped animals ERWIN VOIT, ARON and SEBAUER and others[2] produced, by lack of lime-salts, a change similar to rachitis. In full-grown animals the bones were changed after a long time because of the lack of lime salts in the food, but did not become soft, only thinner (osteoporosis). The attempts to remove the lime-salts from the bones by the addition of lactic acid to the food have led to no positive results (HEITZMANN, HEISS, BAGINSKY [3]). WEISKE, on the contrary, has shown, by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral bodies in the bones might be diminished. On feeding continuously for a long time with a food which yielded an acid ash (cereal grains), WEISKE observed a diminution in the mineral substances of the bones in full-grown herbivora.[4] A few investigators are of the opinion that in rachitis, as in osteomalacia, a solution of the lime-salts by means of lactic acid takes place. This was suggested by the fact that O. WEBER and C. SCHMIDT [5] found lactic acid in the cyst-like, altered bony substance in osteomalacia.

Well-known investigators have disputed the possibility of the lime-

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[1] Pflüger's Arch., **106**.

[2] Zeitschr. f. Biologie, **16**; Aron and Sebauer, Bioch. Zeitschr., 8; A. Baginsky, Arch. f. (Anat. u.) Physiol., 1881.

[3] Heitzmann, Maly's Jahresber., **3**, 229; Heiss, Zeitschr. f. Biologie, **12**; Baginsky, Virchow's Arch., **87**.

[4] See Maly's Jahresber., **22**; also Weiske, Zeitschr. f. physiol. Chem., **20**, and Zeitschr. f. Biologie, **31**.

[5] Cited from v. Gorup-Besanez, Lehrb. de. physiol. Chm., 4. Aufl.

salts being washed from the bones in osteomalacia by means of lactic acid. They have given special prominence to the fact that the lime-salts held in solution by the lactic acid must be deposited on neutralization of the acid by the alkaline blood. This objection is not very important, as the alkaline blood-serum has the property to a high degree of holding earthy phosphates in solution, which fact can be easily proven. The investigations of LEVY [1] contradict the claim as to the solution of the lime-salts by lactic acid in osteomalacia. He found that the normal relation $6PO_4$:10Ca is retained in all parts of the bones in osteomalacia, which would not be the case if the bone-earths were dissolved by an acid. The decrease in phosphate occurs in the same quantitative relation as the carbonate, and according to LEVY, in osteomalacia the exhaustion of the bone takes place by a decalcification in which one molecule of phosphate-carbonate after the other is removed.

In rachitis the quantity of organic matter has been found to vary between 664 and 811 p. m. The quantity of inorganic substance was 189–336 p. m. These figures refer to the dried substance. According to BRUBACHER, rachitic bones are richer in water than the bones of healthy children, and poorer in mineral bodies, especially calcium phosphate. In opposition to rachitis, osteomalacia is often characterized by the considerable amount of fat in the bones, 230–290 p. m., but as a rule the composition varies so much that the analyses are of little value. In a case of osteomalacia, CHABRIE [2] found a larger quantity of magnesium than calcium in a bone. The ash contained 417 p. m. phosphoric acid, 222 p. m. lime, 269 p. m. magnesia, and 86 p. m. carbon dioxide. Other investigators have on the contrary found considerably more calcium than magnesium.

The **tooth-structure** is closely related, from a chemical standpoint, to the bony structure.

Of the three chief constituents of the teeth—dentin, enamel, and cement—the *cement* is to be considered as true bony structure, and as such has already been discussed to some extent. *Dentin* has the same composition as the bony structure, but contains somewhat less water. The organic substance yields gelatin on boiling; but the dental tubes are not dissolved, therefore they cannot consist of collagen. In dentin 260–280 p. m. organic substance has been found. *Enamel* is an epithelium formation containing a large proportion of lime-salts. Corresponding to its character and origin, the organic substance of the enamel does not yield any gelatin. Completely developed enamel contains the least water, the greatest quantity of mineral substances, and is the hardest of all the tissues of the body. In full-grown animals it contains hardly any water, and the quantity of organic substance amounts to only 20–40–68 p. m. The relative amounts of calcium and phosphoric acid are shown by the analyses of HOPPE-SEYLER to be about the same as

[1] Zeitschr. f. physiol. Chem., **19**.

[2] Chabrie, Les phénomènes chim. de l'ossification, Paris, 1895, 65.

in bone-earths. The quantity of chlorine according to him is remarkably high, 0.3–0.5 per cent, while BERTZ [1] found that the ash of enamel was free from chlorine and that dentin was very poor in chlorine.

CARNOT,[2] who has investigated the dentin from elephants, has found 4.3 p. m. calcium fluoride in the ash. In ivory he found only 2 p. m. Dentin from elephants is rich in magnesium phosphate, which is still more abundant in ivory.

GABRIEL found that the quantity of fluorine is very small and amounts to 1 p. m. in ox-teeth. It is no greater in the teeth and enamel than in the bones.[3] The same investigator found that the amount of phosphates is strikingly small in the enamel, and in the teeth considerable lime is replaced by magnesia. This coincides with BERTZ'S findings, that dentin contains twice as much magnesia as the enamel.

According to GASSMANN [4] the teeth among themselves have different composition, and in man the wisdom teeth are poorer in organic substance and richer in lime than the canine teeth. The great tendency of the first to caries is probably explained by this fact. The reason for the degeneration of the teeth is considered by C. RÖSE [5] to be a lack of earthy salts and according to him one finds the best teeth in localities where the drinking water has strong permanent hardness.

IV. THE FATTY TISSUE.

The membranes of the fat-cells withstand the action of alcohol and ether. They are not dissolved by acetic acid or by dilute mineral acids, but are dissolved by artificial gastric juice. They may possibly consist of a substance closely related to elastin. The fat-cells contain, besides fat, a yellow pigment which in emaciation does not disappear so rapidly as the fat; and this is the reason that the subcutaneous cellular tissue of an emaciated corpse has a dark orange-red color. The cells deficient in or nearly free from fat, which remain after the complete disappearance of the latter, seem to have an albuminous protoplasm rich in water. Adipose tissue is rich in a fat-splitting enzyme and in catalases.

The less water the fatty tissue contains the richer it is in fat. SCHULZE and REINECKE [6] found in 1000 parts:

	Water.	Membrane.	Fat.
Fatty tissue of oxen	99.7	16.6	883.7
Fatty tissue of sheep	104.8	16.4	878.8
Fatty tissue of pigs	64.4	13.6	922.0

The fat contained in the fat-cells chiefly consists of triglycerides of stearic, palmitic, and oleic acids. Besides these, especially in the less

[1] See Maly's Jahresber., **30**.
[2] Compt. rend., **114**.
[3] See footnote 4, p. 527.
[4] Zeitschr. f. physiol. Chem., **55**.
[5] Deutsch. Monatsh. f. Zahnheilk., 1908.
[6] Annal. d. Chem. u. Pharm., **142**.

solid kinds of fats, there are glycerides of other fatty acids (see Chapter V). In all animal fats there are besides these, as FR. HOFMANN[1] has shown, also free, non-volatile fatty acids, although in very small amounts.

Human fat is relatively rich in olein, the quantity in the subcutaneous fatty tissue being 70–80 per cent or more.[2] In new-born infants it is poorer in oleic acid than in adults (KNÖPFELMACHER, SIEGERT, JAECKLE); the quantity of olein increases until the end of the first year, when it is about the same as in adults. The composition of the fat in man as well as in different individuals of the same species of animals is rather variable, a fact which is probably dependent upon the food. According to the researches of HENRIQUES and HANSEN the fat of the subcutaneous fatty tissue is richer in olein than that of the internal organs; this has also been observed by LEICK and WINKLER.[3] In animals with a thick subcutaneous fat deposit the outer layers, according to HENRIQUES and HANSEN, are richer in olein than the inner layers. The fat of cold-blooded animals is especially rich in olein. The fat of domestic animals has, according to AMTHOR and ZINK, a less oily consistency and a lower iodine and acetyl equivalent than the corresponding fat of wild animals. Under pathological conditions the fat may have a markedly pronounced variation. The fat of lipoma seems, from JAECKLE's experience, to be poorer in lecithin than other fats.

The properties of fats in general, and the three most important varieties of fat in particular, have already been considered in a previous chapter, hence the formation of the adipose tissue is of chief interest at this time.

The formation of fat in the organism may occur in various ways. The fat of the animal body may consist partly of fat absorbed from the food and deposited in the tissues, and partly of fat formed in the organism from other bodies, such as proteins (?) or carbohydrates.

That the fat from the food which is absorbed in the intestinal canal may be retained by the tissues has been shown in several ways. RADZIEJEWSKI, LEBEDEFF, and MUNK have fed dogs with various fats, such as linseed-oil, mutton-tallow, and rape-seed-oil, and have afterward found the administered fat in the tissues. HOFMANN starved dogs until they appeared to have lost their fat and then fed them upon large quantities of fat and only little proteins. When the animals were killed he found so large a quantity of fat that it could not have been formed from the administered proteins alone, but the greater part must have

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[1] Ludwig-Festschrift, 1874, Leipzig.

[2] See Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature).

[3] Knöpfelmacher, Jahrbuch f. Kinderheilkunde (N. F.), **45** (older literature); Siegert, Hofmeister's Beiträge, **1**; Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature); Henriques and Hansen, Skand. Arch. f. Physiol., **11**; Leick and Winkler, Arch. f. Path. u. Pharm., **48**.

been derived from the fat of the food. PETTENKOFER and VOIT arrived at similar results in regard to the action of the absorbed fats in the organism, though their experiments were of another kind. MUNK found that on feeding with free fatty acids, these are deposited in the tissues, not, however, as such; but they are transforned by synthesis with glycerin into neutral fats on their passage from the intestine into the thoracic duct. The connection between the fat of the food and of the body has also been shown by others, especially by ROSENFELD. CORONEDI and MARCHETTI and in particular WINTERNITZ [1] have recently shown that the iodized fat is taken up in the intestinal tract and deposited in the various organs.

Proteins and carbohydrates are considered as the mother-substances of the fats formed in the organism.

The formation of the so-called *corpse-wax, adipocere,* which consists of a mixture of fatty acids, ammonia, and lime-soaps, from parts of the corpse rich in proteins, is sometimes given as a proof of the *formation of fats from proteins.* The accuracy of this view has, however, been disputed, and many other explanations of the formation of this substance have been offered. According to the experiments of KRATTER and K. B. LEHMANN, it seems as if it were possible by experimental means to convert animal tissue rich in proteins (muscles) into adipocere by the continuous action of water. Irrespective of this, SALKOWSKI has shown that in the formation of adipocere the fat itself takes part, in that the olein decomposes with the formation of solid fatty acids, still it must be considered that lower organisms undoubtedly take part in its formation. The production of adipocere as a proof of the formation of fat from proteins is disputed by many investigators for this and other reasons.

Fatty degeneration has been considered as another proof of the formation of fat from proteins. From the investigations of BAUER on dogs and LEO on frogs it was assumed that, at least in acute poisoning by phosphorus, a fatty degeneration, with the formation of fat from proteins, takes place. PFLÜGER has raised such strong arguments against the older researches as well as the more recent one of POLIMANTI, who claims to have shown the formation of fat from proteins in phosphorus poisoning, that we cannot consider the formation of fat as conclusively proven. Recent investigations of ATHANASIU, TAYLOR, SCHWALBE, and others, especially of ROSENFELD,[2] have shown that probably no new

[1] Coronedi and Marchetti, cited by Winternitz, Zeitschr. f. physiol. Chem., 24. A review of the literature on fat formation may be found in Rosenfeld, Fettbildung, in Ergebnisse der Physiologie, **1**, Abt. 1.

[2] Bauer, Zeitschr. f. Biologie, **7**; Leo, Zeitschr. f. physiol. Chem., **9**; Polimanti, Pflüger's Arch., **70**; Pflüger, *ibid.*, **51** (literature on the formation of fat from protein) and **71**; Athanasiu, *ibid.*, **74**; Taylor, Journ. Exp. Medicine, **4**; see also footnote 1, p. 368.

formation of fat from protein took place, but rather a fat migration (Rosenfeld).

Another more direct proof of the formation of fat from proteins has been given by Hofmann. He experimented with fly-maggots. A number of these were killed and the quantity of fat determined. The remainder were allowed to develop in blood whose proportion of fat had been previously determined, and after a certain time they were killed and analyzed. He found in them from seven to eleven times as much fat as was contained in the maggots first analyzed and the blood taken together. Pflüger [1] has made the objection that a considerable number of lower fungi develop in the blood under these conditions, in whose cell-body fats and carbohydrates are formed from the different constituents of the blood and their decomposition products, and that these serve as food for the maggots.

Weinland [2] has observed the formation of higher non-volatile fatty acids in the Calliphora larvae when they were rubbed to a homogeneous paste after the addition of Witte's peptone. This experiment shows a formation of fat from protein, but cannot be considered as quite conclusive.

As a more convincing proof of fat formation from proteins, the investigations of Pettenkofer and Voit are often quoted. These investigators fed dogs with large quantities of meat containing the least possible proportion of fat, and found all of the nitrogen in the excreta, but only a part of the carbon. As an explanation of these conditions it has been assumed that the protein of the organisms splits into a nitrogenized and a non-nitrogenized part, the former changing into the nitrogenized final product, urea, and like products, and the other part, on the contrary, being retained in the organism as fat (Pettenkofer and Voit).

Pflüger has arrived at the following conclusion by an exhaustive criticism of Pettenkofer and Voit's experiments and a careful recalculation of their balance-sheet; that these very meritorious investigations, which were continued for a series of years, were subject to such great defects that they are not conclusive as to the formation of fat from proteins. He especially emphasizes the fact that these investigators started from a wrong assumption as to the elementary composition of the meat, and that the quantity of nitrogen assumed by them was too low and the quantity of carbon too high. The relation of nitrogen to carbon in meat poor in fat was assumed by Voit to be as 1:3.68, while according to Pflüger it is 1:3.22 for fat-free meat after deducting the glycogen, and according to Rubner 1:3.28 without deducting the glycogen. On recalculation of the figures, using these coefficients, Pflüger

[1] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie, 1, Abt. 1.

[2] Zeitschr. f. Biol., 51.

has arrived at the conclusion that the assumption as to the formation of fat from proteins finds no support in these experiments.

In opposition to these objections, E. VOIT and M. CREMER have made new feeding experiments to show the formation of fat from proteins, but the proof of these recent investigations has been disputed by PFLÜGER. On feeding a dog on meat poor in fat (containing a known quantity of ether extractives, glycogen, nitrogen, water, and ash), KUMAGAWA[1] could not prove the formation of fat from protein. According to him the animal body under normal conditions has not the power of forming fat from protein.

Several French investigators, especially CHAUVEAU, GAUTIER, and KAUFMANN,[2] consider the formation of fat from proteins as positively proven. KAUFMANN has recently substantiated this view by a method which will be spoken of in detail in Chapter XVIII, in which he studied the nitrogen elimination and the respiratory gas exchange in conjunction with the simultaneous formation of heat.

As we are agreed that carbohydrates and glycogen, as well as sugar, can be formed from proteins, the fact cannot be denied that possibly an indirect formation of fat from proteins, with a carbohydrate as an intermediate step, can take place. The possibility of a direct fat formation from proteins without the carbohydrate as intermediary must also be generally admitted, although such a formation has not been conclusively proven.

According to CHAUVEAU and KAUFMANN, in the direct formation of fat from proteins, the fat is formed besides urea, carbon dioxide, and water, as an intermediary product in the oxidation of the proteins, while GAUTIER considers the formation of fat from proteins as a cleavage without the taking up of oxygen. If fat is formed from protein in the animal body, then such formation is not a splitting off of fat from the proteins, but rather a synthesis from primarily formed cleavage products of proteins which are poor in carbon.

The *formation of fat from carbohydrates* in the animal body was first suggested by LIEBIG. This was combated for some time, and until lately it was the general opinion that a direct formation of fat from carbohydrates not only had not been proven, but also that it was improbable. The undoubtedly great influence of the carbohydrates on the formation of fat as observed and proven by LIEBIG was explained by the statement that the carbohydrates were consumed instead of the absorbed fat or that derived from the proteins, hence they have a sparing action on the fat. By means of a series of nutrition experiments

[1] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie, **1**, Abt. 1.

[2] Kaufmann, Arch. de physiol., (5) 8, where the works of Chauveau and Gautier are cited.

with foods especially rich in carbohydrates LAWES and GILBERT, SOXHLET, TSCHERWINSKY, MEISSL, and STROMER (on pigs), B. SCHULTZE, CHANIEWSKI, E. VOIT and C. LEHMANN (on geese), I. MUNK and RUBNER and LUMMERT [1] (on dogs) apparently prove that a direct formation of fat from carbohydrates does actually occur. The processes by which this formation takes place are still unknown. As the carbohydrates do not contain such complicated carbon chains as the fats, the formation of fat from carbohydrates must consist of a synthesis, in which the group CHOH is converted into CH_2; hence a reduction must occur.

After feeding with very large quantities of carbohydrates the relation between the inspired oxygen and the expired carbon dioxide, i.e., the respiratory quotient $\frac{CO_2}{O}$, was found greater than 1 in certain cases (HANRIOT and RICHET, BLEIBTREU, KAUFMANN, LAULANIÉ [2]). This is explained by the assumption that the fat is formed from the carbohydrate by a cleavage setting free carbon dioxide and water without taking up oxygen. This increase in the respiratory quotient also depends in part on the increased combustion of the carbohydrate.

When food contains an excess of fat the superfluous amount is stored up in the fatty tissue, and on partaking of food deficient in fat this accumulation is quickly exhausted; and it is very probable that the lipase is of importance here, as LOEVENHART [3] has found that all over the body where fat is deposited in large amounts lipase also occurs in considerable amounts. There is perhaps not one of the various tissues that decreases so much in starvation as the fatty tissue. The organism, then, possesses in this tissue a depot where there is stored during proper alimentation a nutritive substance of great importance in the development of heat and vital force, which substance, on insufficient nutrition, is given up as may be needed. On account of their low conducting power, the fatty tissues become of great importance in regulating the loss of heat from the body. They also serve to fill cavities and act as a protection and support to certain internal organs.

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[1] Lawes and Gilbert, Phil. Transactions, 1859, part 2; Soxhlet, see Maly's Jahresber., **11**, 51; Tscherwinsky, Landwirthsch. Versuchsstaat, **29** (cited from Maly's Jahresber., **13**;) Meissl and Stromer, Wien. Sitzungsber., 88, Abt. 3; Schultze, Maly's Jahresber., **11**, 47; Chaniewski, Zeitschr. f. Biologie, **20**; Voit and Lehmann, see C. v. Voit, Sitzungsber. d. k. bayer. Akad. d. Wissensch., 1885; I. Munk, Virchow's Arch., **101**; Rubner, Zeitschr. f. Biologie, **22**; Lummert, Pflüger's Arch., **71**.

[2] Hanriot and Richet, Annal. de Chim. et de Phys. (6), **22**; Bleibtreu, Pflüger's Arch., **56** and **85**; Kaufmann, Arch. de Physiol. (5), 8; Laulanié, *ibid.*, 791.

[3] Amer. Journ. of Physiol., **6**.

CHAPTER XI.

MUSCLES.

STRIATED MUSCLES.

In the study of the muscles the chief problem for physiological chemistry is to isolate their different morphological elements and to investigate each element separately. By reason of the complicated structure of the muscles this has been thus far almost impossible, and we must be satisfied at the present time with a few microchemical reactions in the investigation of the chemical composition of the muscular fibres.

Each muscle-tube and each muscle-fibre consists of a sheath, the SARCOLEMMA, which seems to be composed of a substance similar to elastin, and containing a large proportion of *protein*. This last, which in life posesses the power of contractility, has in the inactive muscle an alkaline reaction, or, more correctly speaking, an amphoteric reaction with a predominating action on red litmus paper. RÖHMANN found that the fresh, inactive muscle shows an alkaline reaction with red lacmoid, and an acid reaction with brown turmeric. From the effect of various acids and salts on these coloring-matters he concludes that the alkalinity of the fresh muscle with lacmoid is due to sodium bicarbonate, diphosphate, and probably also to an alkaline combination of protein bodies, and the acid reaction with turmeric, on the contrary, to monophosphate chiefly. The dead muscle has an acid reaction, or, more correctly, the acidity with turmeric increases on the decease of the muscle, and the alkalinity with lacmoid decreases. The difference depends on the presence of a larger quantity of monophosphate in the dead muscle, and according to RÖHMANN free lactic acid is found in neither the one case nor the other.[1]

If the somewhat disputed statements relative to the finer structure of the muscles are disregarded, one can differentiate in the striated muscles between the two chief components, the doubly refracting—*anisotropous*—and the singly refracting—*isotropous*—substance. If the muscular fibres are treated with reagents which dissolve proteins,

[1] The various reports in regard to the reaction of the muscles and the cause thereof are conflicting. See Röhmann, Pflüger's Arch., **50** and **55**; Heffter, Arch. f. exp. Path. u. Pharm., **31** and **38**. These references contain the pertinent literature.

such as dilute hydrochloric acid, soda solution, or gastric juice, they swell greatly and break up into "BOWMAN'S *disks*." By the action of alcohol, chromic acid, boiling water, or in general such reagents as cause a shrinking, the fibres split longitudinally into fibrils; and this behavior shows that several chemically different substances of various solubilities enter into the construction of the muscular fibres.

The protein myosin is generally considered as the chief constituent of the diagonal disks, while the isotropous substance contains the chief mass of the other proteins of the muscles as well as the chief portion of the extractives. According to the observations of DANILEWSKY, confirmed by J. HOLMGREN,[1] myosin may be completely extracted from the muscle without changing its structure, by means of a 5-per cent solution of ammonium chloride, which fact conflicts with the above view. DANILEWSKY claims that another protein-like substance, insoluble in ammonium chloride and only swelling up therein, enters essentially into the structure of the muscles. The proteins, which form the chief part of the solids of the muscles, are of the greatest importance.

Proteins of the Muscles.

Like the blood which contains a fluid, the blood-plasma, which spontaneously coagulates, separating fibrin and yielding blood-serum, so also the living muscle, at least of cold-blooded animals, contains, as first shown by KÜHNE, a spontaneously coagulating liquid, the muscle plasma, which coagulates quickly, separating a protein body, myosin, and yielding also a serum. That liquid which is obtained by pressing the living muscle is called *muscle-plasma*, while that obtained from the dead muscle is called *muscle-serum*. These two fluids contain different protein bodies.

Muscle-plasma was first prepared by KÜHNE from frog-muscles, and later by HALLIBURTON, according to the same method, from the muscles of warm-blooded animals, especially rabbits. The principle of this method is as follows: The blood is removed from the muscles immediately after the death of the animal by passing through them a strongly cooled common-salt solution of 5–6 p. m. Then the muscles are quickly cut and immediately thoroughly frozen so that they can be ground in this state to a fine mass—"muscle-snow." This pulp is strongly pressed in the cold, and the liquid which exudes is called muscle-plasma. According to v. FÜRTH[2] this cooling or freezing is not necessary. It

[1] Danilewsky, Zeitschr. f. physiol. Chem., **7**; J. Holmgren, Maly's Jahresber., **23**.

[2] See Kühne, Untersuchungen über das Protoplasma (Leipzig, 1864), 2; Halliburton, Journ. of Physiol., 8; v. Fürth, Arch. f. exp. Path. u. Pharm., **36** and **37**; Hofmeister's Beiträge, **3**, and Ergebnisse der Physiologie, **1**, Abt. 1; Stewart and Sollmann, Journ. of Physiol., **24**.

is sufficient to extract the muscle free from blood, as above directed, with a 6 p. m. common-salt solution.

Muscle-plasma forms a yellow to brownish-colored fluid with an alkaline reaction. It varies in different animals. Muscle-plasma from the frog spontaneously coagulates slowly at a little above 0° C., but more quickly at the temperature of the body. Muscle-plasma from mammals coagulates slowly, according to v. FÜRTH, even at the temperature of the room, though only slightly, and it can hardly be considered as a process comparable with the coagulation of the blood. Indeed the question may be asked whether a true muscle-plasma does exist in warm-blooded animals, or whether the fluid obtained from such muscles exactly represents the plasma of the living muscle. According to KÜHNE and v. FÜRTH the reaction remains alkaline during coagulation, while HALLIBURTON, STEWART and SOLLMANN find that it becomes acid. Earlier investigators held that the clot consists of a globulin called myosin, while v. FÜRTH claims that it consists of two coagulated proteins, myosin-fibrin and myogen-fibrin.

The study of the proteins of the muscles, as well as their nomenclature, has changed markedly in the last few years, and it is questionable whether an essential difference exists between the proteins of the muscle-plasma and the muscle-serum of warm-blooded animals. Nevertheless it is necessary to separately discuss the proteins of the dead muscle as well as those of the muscle-plasma.

The *proteins of the dead muscle* are in part soluble in water or dilute salt solutions, and in part are insoluble therein. Myosin and musculin and also myoglobulin and myoalbumin, which exist to a very slight extent and are perhaps only derived from the remaining lymph, belong to the first group, and the stroma substances of the muscle-tubes belong to the second group.

Myosin was first discovered by KÜHNE, and constitutes the principal mass of the soluble proteins of the dead muscle. It is generally considered as the most essential coagulation product of muscle-plasma. The name myosin KÜHNE also gives to the mother-substance of the plasma-clot, and this mother-substance forms, according to certain investigators, the chief mass of contractile protoplasm. The findings as to the occurrence of myosin in other organs besides the muscles require further confirmation. The quantity of myosin in the muscles of different animals varies, according to DANILEWSKY,[1] between 30 and 110 p. m.

Myosin, as obtained from dead muscles, is a globulin whose elementary composition, according to CHITTENDEN and CUMMINS,[2] is, on an average, the following: C 52.28, H 7.11, N 16.77, S 1.27, O 22.03 per cent. If the

[1] Zeitschr. f. physiol. Chem., 7.

[2] Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 3, 115.

myosin separates as fibres, or if a myosin solution with a minimum quantity of alkali is allowed to evaporate to a gelatinous mass on a microscope-slide, doubly refracting myosin may be obtained. Myosin has the general properties of the globulins. It is insoluble in water, but soluble in dilute saline solutions as well as in dilute acids or alkalies, which readily convert it into albuminates. It is completely precipitated upon saturation with NaCl, also by $MgSO_4$, in a solution containing 94 per cent of the salt with its water of crystallization (HALLIBURTON). The precipitated myosin readily becomes insoluble. Like fibrinogen it coagulates at 56° C. in a solution containing common salt, but differs from it, since under no circumstances can it be converted into fibrin. The coagulation temperature, according to CHITTENDEN and CUMMINS, not only varies for myosins of different origin, but also for the same myosin in different salt solutions.

Myosin may be prepared in the following way, as suggested by HALLIBURTON: The muscle is first extracted by a 5-per cent magnesium-sulphate solution. Tne filtered extract is then treated with magnesium sulphate in substance until 100 cc. of the liquid contain about 50 grams of the salt. The so-called paramyosinogen or musculin separates. The filtered liquid is then treated with magnesium sulphate until each 100 cc. of the liquid hold 94 grams of the salt in solution. The myosin which now separates is filtered off, dissolved in water by aid of the retained salt, precipitated by diluting with water, and, when necessary, purified by redissolving in dilute salt solution and precipitating with water.

The older and perhaps the usual method of preparation consists, according to DANILEWSKY,[1] in extracting the muscle with a 5–10-per cent ammonium-chloride solution, precipitating the myosin from the filtrate by strongly diluting with water, and redissolving the precipitate in ammonium-chloride solution, and the myosin obtained from this solution is reprecipitated either by diluting with water or by removing the salt by dialysis.

Musculin,[2] called PARAMYOSINOGEN by HALLIBURTON, and MYOSIN by v. FÜRTH, is a globulin which is characterized by its low coagulation temperature, in frogs below 40°, in mammalia 42–48°, and in birds about 51° C., and which may vary in different species of animals. It is more easily precipitated than myosin by NaCl or $MgSO_4$ (50 per cent salt, including water of crystallization). According to v. FÜRTH it is precipitated by ammonium sulphate with a concentration of 12–24 per cent salt. If the dead muscle is extracted with water a part of the musculin

[1] Zeitschr. f. physiol. Chem., 5, 158.

[2] As we have up to the present no conclusive basis for the identity of the globulins called myosin and paramyosinogen, and also as the use of the name myosin for the last-mentioned substance may readily cause confusion, the author does not feel justified in dropping the old name musculin (Nasse).

goes into solution, and may be precipitated therefrom by carefully acidifying. It separates from a dilute salt solution on dialysis. Musculin readily passes into an insoluble modification which v. FÜRTH calls *myosin fibrin*. Musculin is called myosin by v. FÜRTH, as he considers it nothing but myosin. As musculin has a lower coagulation temperature and has other precipitating properties for neutral salts than the older substance called myosin, it is difficult to accept this view.

Myoglobulin. After the separation of the musculin and the myosin from the salt extract of the muscle by means of $MgSO_4$, the myoglobulin may be precipitated by saturating the filtrate with the salt. It is similar to serglobulin, but coagulates at 63° C. (HALLIBURTON). *Myoalbumin*, or muscle-albumin, seems to be identical with seralbumin (seralbumin α, according to HALLIBURTON), and probably originates only from the blood or the lymph. Proteoses and peptones do not seem to exist in the fresh muscles.

After the complete removal from the muscle of all protein bodies which are soluble in water and ammonium chloride, an insoluble protein remains which only swells in ammonium-chloride solution, and which forms with the other insoluble constituents of the muscular fibre the "*muscle-stroma.*" According to DANILEWSKY the amount of such stroma substance is connected with the muscle activity. He maintains that the muscles contain a greater amount of this substance, compared with the myosin present, when the muscles are quickly contracted and relaxed, the correctness of which report has recently been disputed by SAXL.[1]

According to J. HOLMGREN,[2] this stroma substance does not belong to either the nucleoalbumin or the nucleoprotein group. It is not a glucoproteid, as it does not yield a reducing substance when boiled with dilute mineral acids. It is very similar to the coaguable proteins, and dissolves in dilute alkalies, forming an albuminate. The elementary composition of this substance is nearly the same as that of myosin. There is no doubt that the insoluble substances, myofibrin and myosin fibrin, which are formed, according to v. FÜRTH, in the coagulation of the plasma, also occur among the stroma substances. When the muscles are previously extracted with water, the stroma substances also contain a part of the myosin hereby made insoluble. The observations of SAXL on rabbits' muscles agree with this view that the fresh muscle after work contains 11.5–21.6 per cent of the total protein in an insoluble form, while the muscle after rigor mortis contains on the contrary 71.5–73.2 per cent.

To the proteins insoluble in water and neutral salts belongs the *nucleoprotein* detected by PEKELHARING, which occurs as traces and is soluble in faintly alkaline water, and which probably originates from

[1] Hofmeister's Beiträge, 9.

[2] See footnote 1, p. 539.

the muscle nuclei. According to BOTTAZZI and DUCCESCHI [1] the heart muscle is richer in nucleoprotein than the skeletal muscle.

Muscle-syntonin, which may be obtained by extracting the muscles with hydrochloric acid of 1 p. m., and which, according to K. MÖRNER, is less soluble and has a greater aptitude to precipitate than other acid albumins, seems not to occur preformed in the muscles. HEUBNER'S [2] *mytolin* is modified muscle-proteid, chiefly myosin, which has lost a part of its sulphur by the action of alkali.

Proteins of the Muscle-plasma. As above stated, myosin was ordinarily considered as the coagulated modification of a soluble protein existing in the muscle-plasma. As in blood-plasma there is present a mother-substance of fibrin, fibrinogen, so also there exists in the muscle plasma a mother-substance of myosin, a soluble myosin or a *myosinogen.* This body has not thus far been isolated with certainty. HALLIBURTON, who has detected in the muscles an enzyme-like substance, "*myosin ferment,*" which is related to fibrin ferment but is not identical with it, has also found that a solution of purified myosin, in dilute salt solution (5 per cent $MgSO_4$), and sufficiently diluted with water, coagulates after a certain time, and at the same time becomes acid, and a typical myosin-clot separates. This coagulation, which is accelerated by warming or by the addition of myosin ferment, is, according to HALLIBURTON, a process analogous to the coagulation of the muscle-plasma. According to this same investigator, myosin when dissolved in water by the aid of a neutral salt is reconverted into myosinogen, while after diluting with water myosin is again produced from the myosinogen. The musculin (paramyosinogen) is carried down, according to HALLIBURTON, with the myosin-clot, but has nothing to do with the coagulation, as the myosin-clot also forms in the absence of musculin, and this last is not changed into myosin.

Besides the traces of globulin and albumin, which perhaps do not belong to the muscle-plasma, there occur in mammals, according to v. FÜRTH, two proteins, namely, musculin (myosin according to v. FÜRTH) and myogen.

MUSCULIN (NASSE) = paramyosinogen (HALLIBURTON) = myosin (v. FÜRTH) forms about 20 per cent of the total proteins of the muscle-plasma of rabbits. Its properties have already been given, and it is sufficient to remark that its solutions become cloudy on standing, and a precipitate of *myosin fibrin* occurs, which is insoluble in salt solutions.

Myogen, or MYOSINOGEN (HALLIBURTON), forms the chief mass, 75–80 per cent, of the proteins of rabbit muscle-plasma. It does not separate from its solutions on dialysis and is not a true globulin, but

[1] Pekelharing, Zeitschr. f. physiol. Chem., **22**; Bottazzi and Ducceschi, Centrabl. f. Physiol., **12**.

[2] Arch. f. exp. Pathol. u. Pharm., **53**.

a protein *sui generis*. It coagulates at 55–65° C. and is precipitated in the presence of 26–40 per cent ammonium sulphate. Myogen solutions are precipitated by acetic acid only in the presence of some salt. It is converted into an albuminate by alkalies, this albuminate being precipitable by ammonium chloride. Myogen passes spontaneously, especially with higher temperatures as well as in the presence of salt, into an insoluble modification, *myogen fibrin*. A protein, coagulating at 30–40° C., *soluble myogen fibrin*, is produced as a soluble intermediate step. This substance occurs to a considerable extent in native frog-muscle plasma. It does not always occur in the muscle-plasma of warm-blooded animals, and when it does it is present only to a slight extent. It can be separated by precipitating with salt or by diffusion. HALLIBURTON'S assumption as to the action of a special myosin ferment has not sufficient basis, according to v. FÜRTH, nor has the often-admitted analogy with the coagulation of the blood. The difference between the musculin and the myogen in their becoming insoluble is that the musculin passes into myosin fibrin without any soluble intermediate steps.

Myogen may be prepared, according to v. FÜRTH, by heating, for a short time, the dialyzed and filtered plasma to 52° C., separating it in this way from the rest of the musculin. The myogen exists in the new filtrate and can be precipitated by ammonium sulphate. The musculin may also be removed by adding 28 per cent ammonium sulphate and then precipitating the myogen from the filtrate by saturating with the salt.

STEWART and SOLLMANN admit of only two soluble proteins in the muscles. One is the paramyosinogen, which is the same as v. FÜRTH'S myosin+the soluble myogen fibrin. The other they call myosinogen, which corresponds to v. FÜRTH'S myogen or to HALLIBURTON'S myosinogen+myoglobulin. It is a typical globulin which coagulates at 50–60° C. The paramyosinogen as well as the myosinogen is readily converted into an insoluble modification, myosin. The myosin of the above investigators is the same as v. FÜRTH'S myosin fibrin+myogen fibrin, and corresponds, it seems, also to myosin mixed with paramyosinogen (HALLIBURTON). STEWART and SOLLMANN differ from HALLIBURTON in considering that paramyosinogen also coagulates and is converted into myosin. According to them myosin is also insoluble in a NaCl solution.

The views of the various investigators differ so essentially and the nomenclature is so complicated (three different things are designated by the name myosin) that it is extremely difficult to give any correct review of the various opinions.[1] Thorough investigations on this subject are very necessary.

[1] For these reasons the author is not sure whether he has understood and correctly given the work of the different investigators.

Myoproteid is a protein found by v. FÜRTH in the plasma from fish-muscles. It does not coagulate on boiling, is precipitated by acetic acid, and is considered as a compound protein by v. FÜRTH.

In connection with v. FÜRTH's work, PRZIBRAM has carried on investigations on the occurrence of muscle-proteins in various classes of animals. The myosin (v. FÜRTH) and myogen occur in all classes of vertebrates; the myogen is always absent in the invertebrates. Myoproteid occurs, at least in considerable quantity, only in fishes. In the muscle after cutting the nerve, STEYRER [1] found somewhat more musculin and less myogen in the muscle-juice than in the normal muscle.

Muscle-pigments. There is no question that the red color of the muscles, even when completely freed from blood, depends in part on hæmoglobin. K. MÖRNER has shown that muscle-hæmoglobin is not quite identical with blood-hæmoglobin. The statement of MACMUNN that in the muscles another pigment occurs which is allied to hæmochromogen, and called *myohæmatin* by him, has not been substantiated, at least for muscles of higher animals (LEVY and MÖRNER [2]). MACMUNN claims that myohæmatin occurs in the muscles of insects, which do not contain any hæmoglobin. The reddish-yellow coloring-matter of the muscles of the salmon has been little studied.

Various enzymes have been found in the muscles. To these belong (besides traces of fibrin ferment and myosin ferment) the *catalases* and *oxidases*, which occur only to a slight extent. The disputed glycolytic enzyme (Chapter VIII), whose nature is unknown, probably belongs to the oxidases. An amylolytic and a proteolytic enzyme (HEDIN and ROWLAND [3]) have also been found, and the hydrolytic and oxidizing enzymes (Chapter XV) active in the formation and destruction of uric acid are also present.

Extractive Bodies of the Muscles.

The *nitrogenous extractives* consist chiefly of *creatine*, on an average of 1–5 p. m. in the fresh muscles containing water, also the *purine bases*, *hypoxanthine* and *xanthine*, besides *guanine* and *carnine*, but chiefly hypoxanthine. The purine bases probably do not occur as such, but as complex combinations. The quantity of nitrogen as purine bases amounts, according to BURIAN and HALL, in the fresh flesh of the horse, ox, and calf to 0.55, 0.63, and 0.71 p. m. respectively, or 1.3–1.7 p. m. calculated as

[1] Przibram, Hofmeister's Beiträge, **2**; Steyrer, *ibid.*, **4**.

[2] See MacMunn Phil. Trans. of Roy. Soc., **177**, part 1, Journ. of Physiol., 8, and Zeitschr. f. physiol. Chem., **13**; Levy, *ibid.*, **13**; K. Mörner, Nord. Med. Archiv, Festband, 1897, and Maly's Jahresber., **27**.

[3] Zeitschr. f. physiol. Chem., **32**.

hypoxanthine. In the embryonic ox-muscles, KOSSEL[1] found more guanine than hypoxanthine. The purine bases are produced in the muscles themselves, and their production, which also takes place while at rest, is greatly increased during work (BURIAN[2]).

Among the apparently habitually occurring nitrogenous extractives, we should also mention *phosphocarnic acid* as well as *inosinic acid*, which is perhaps allied to it, *carnosine*, *carnitine*, and perhaps also other bodies which have recently been found in meat extract and which will be mentioned later.

Among the extractive substances is also found the acid noticed by LIMPRICHT in the flesh of certain cyprinidea, namely, the nitrogenized *protic acid*, while the *isocreatinine* found by J. THESEN in fish-flesh is nothing but impure creatinine, according to POULSSON, SCHMIDT and KORNDÖRFER.[3] *Uric acid*, *urea*, *taurine*, and *leucine* are found as traces in the muscles, in certain cases only in a few species of animals. In regard to the amounts of these different extractives in the muscles, KRUKENBERG and WAGNER[4] have shown that they vary greatly in different animals. A large quantity of urea is found in the muscles of the shark and ray; uric acid is found in alligators; taurine in cephalopoda; *glycocoll* in gasteropoda, and *creatinine* especially in fishes. The reports are very contradictory in regard to the occurrence of urea in the muscles of higher animals. According to the investigations of KAUFMANN and SCHÖNDORFF, confirmed by BRUNTON-BLAIKIE,[5] urea is a regular constituent of the muscles, although M. NENCKI and KOWARSKI dispute this.

The purine bases, with the exception of carnine, have been treated on pages 184–188, and therefore among the extractive bodies we will first consider the creatine.

Creatine, $C_4H_9N_3O_2$, $C{<}^{NH_2}_{N(CH_3).CH_2COOH}{=}NH$, or methyl-guanidine-acetic acid, occurs in the muscles of vertebrate animals in variable amounts, 1.4–5 p. m., in different species; the largest quantity is found in birds. It is also found in the brain, blood, transudates, amniotic fluid, and sometimes also in the urine. Creatine may be prepared synthetically from cyanamide and sarcosine (methylglycocoll). On boiling with baryta-water it decomposes, with the addition of water, and yields urea, sarcosine, and certain other products. Because of this behavior several investiga-

[1] Burian and Hall, Zeitschr. f. physiol. Chem., **38**; Kossel, *ibid.*, 8, 408.

[2] *Ibid.*, **43**.

[3] See Limpricht, Annal. d. Chem. u. Pharm., **127**, and Thesen, Zeitschr. f. physiol. Chem., **24**; Poulsson, Arch. f. exp. Path. u. Pharm., **51**; Schmidt and Korndörfer, *ibid.*, **51**.

[4] Zeitschr. f. Biologie, **21**; see also M. Henze, Zeitschr. f. physiol. Chem., **43**; Mendel, Hofmeister's Beiträge, **5**; Kelly, *ibid.*, 5.

[5] Kaufmann, Arch. de Physiol. (5), **6**; Schöndorff, Pflüger's Arch., **62**; Nencki and Kowarski, Arch. f. exp. Path. u. Pharm., **36**; Brunton-Blaikie, Journ. of Physiol., **23**, Supplement.

tors consider creatine as a step in the formation of urea in the organism. On boiling with acids, creatine is easily converted, with the elimination of water, into the corresponding anhydride, creatinine, $C_4H_7N_3O$, which is retransformed into creatine by the action of alkali.

The question as to the relation of creatine to creatinine within the animal body is disputed, and is intimately related to the question as to the rôle of the creatinine in protein metabolism. As this question is best discussed in connection with creatinine and its elimination in the urine (Chapter XV), we will here only discuss the direct relation of creatine to the muscles and its metabolism.

Of special interest in this regard, besides the relation between creatine and muscle work which will be discussed below, is the question as to the occurrence of free or combined creatine in the muscle. URANO by the aid of dialysis experiments has shown the probability that the creatine does not exist free in the muscle, but as a labile, non-dialyzable combination. Nevertheless GOTTLIEB and STANGASSINGER claim by various researches to have shown in the autolysis of muscles and other organs that creatine is first formed and then first changed into creatinine by special bodies of an enzymotic nature, and then destroyed. SEEMANN [1] indeed claims, by an autolysis of three months' duration, to have obtained two to three times as much creatinine directly from the muscle and after the addition of creatinine-free-gelatin four times as much, which is an argument against the enzymotic destruction of creatinine in autolysis, and he admits of the formation of creatine (or creatinine) from protein. The autolytic experiments of ROTHMANN also indicate the formation of creatine from a preliminary body, and the recent experiments of VAN HOOGENHUYZE and VERPLOEGH make the enzymotic transformation of creatine and creatinine probable. MELLANBY positively denies the re-formation of creatine as well as its destruction in autolysis entirely free from bacteria. It is hard to draw positive conclusions from experiments with autolysis. The transfusion experiments of GOTTLIEB and STANGASSINGER [2] with the kidneys and livers of dogs not only point to the ability of these organs to decompose creatine, but also for a re-formation of creatine in the liver. Further investigations are still very necessary.

Opinions are not unanimous in regard to the organ where creatine is formed. From recent investigations it is concluded that the liver plays an important part therein. Other organs, especially the muscles,

[1] Urano, Hofmeister's Beiträge, **9**; Gottlieb and Stangassinger, Zeitschr. f. physiol. Chem., **52** (and **55**); Stangassinger, *ibid.*, **55**; Seemann, Zeitschr. f. Biol., **49**; Rothmann, Zeitschr. f. physiol. Chem., **57**; v. Hoogenhuyze and Verploegh, *ibid.*, **57**; Mellanby, Journ. of Physiol., **36**.

[2] Zeitschr. f. physiol. Chem., **55**.

are also included. According to MELLANBY the creatinine is probably formed in the liver and transformed into creatine in the muscles and there deposited as such.

Creatine crystallizes in hard, colorless, monoclinic prisms which lose their water of crystallization at 100° C. It is soluble in 74 parts of water at the ordinary temperature, and in 9419 parts absolute alcohol. It dissolves more easily with the aid of heat. Its watery solution has a neutral reaction. Creatine is not dissolved by ether. If a creatine solution is boiled with precipitated mercuric oxide, this is reduced, especially in the presence of alkali, to mercury and oxalic acid, and the foul-smelling methyluramine (methylguanidine) is developed. A solution of creatine in water is not precipitated by basic lead acetate, but gives a white, flaky precipitate with mercurous nitrate if the acid reaction is neutralized. When boiled for an hour with dilute hydrochloric acid, creatine is converted into creatinine, and may be identified by its reactions. On boiling with formaldehyde it can be transformed into dioxymethylenecreatinine, which crystallizes readily (JAFFÉ [1]).

The preparation and detection of creatine is best accomplished by the following method of NEUBAUER,[2] which was first used in the preparation of creatine from muscles: Finely cut meat is extracted with an equal weight of water at 50–55° C. for 10–15 minutes, pressed, and extracted again with water. The proteins are removed from the united extracts so far as possible by coagulation at boiling heat, the filtrate precipitated by the careful addition of basic lead acetate, the lead removed from this filtrate by H_2S, and the solution then carefully concentrated to a small volume. The creatine, which crystallizes in a few days, is collected on a filter, washed with alcohol of 88 per cent, and purified, when necessary, by recrystallization. The quantitative estimation of creatine is performed by transforming it into creatinine (see Chapter XV).

Carnine, $C_7H_8N_4O_3 + H_2O$, is one of the substances found by WEIDEL in American meat extract. It has also been found by KRUKENBERG and WAGNER in frog muscles and in the flesh of fishes, and by POUCHET in the urine. Carnine, which may be transformed into hypoxanthine by oxidation is, according to HAISER and WENZEL,[3] probably only an equimolecular mixture of hypoxanthine and a pentoside, $C_{10}H_{12}N_4O$-, called *inosine,* which is crystalline, and which readily splits into hypoxanthine and pentose by the action of acid.

Carnine has been obtained as a white crystalline mass. It dissolves

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[1] Ber. d. d. Chem. Gesellsch., 35.

[2] Zeitschr. f. analyt. Chem., 2 and 6.

[3] Weidel, Annal. d. Chem. u. Pharm., 158; Krukenberg and Wagner, Sitzungsber. d. Würzb. phys.-med. Gesellsch., 1883; Pouchet, cited from Neubauer-Huppert, Analyse des Harnes, 10. Aufl., 335; Haiser and Wenzel, Monatsch. f. Chem., 29.

with difficulty in cold water, but more readily in warm. It is insoluble in alcohol and ether. It dissolves in warm hydrochloric acid and yields a salt crystallizing in shining needles, which gives a double compound with platinum chloride. Its watery solution is precipitated by silver nitrate, but this precipitate is dissolved neither by ammonia nor by warm nitric acid. Carnine does not give the so-called WEIDEL's xanthine reaction. Its watery solution is precipitated by basic lead acetate; but the lead compound may be dissolved on boiling.

Carnine is prepared by the following method: The meat extract diluted with water is completely precipitated by baryta-water. The filtrate is precipitated by basic lead acetate, the lead precipitate boiled with water, filtered while hot, and sulphuretted hydrogen passed through the filtrate. Remove the lead sulphide from the filtrate and concentrate strongly. The concentrated solution is now completely precipitated with silver nitrate, the precipitate washed free from silver chloride by ammonia, and the carnine silver oxide suspended in water and treated with sulphuretted hydrogen.

Carnosine, $C_9H_{14}N_4O_3$, has been isolated by GULEWITSCH and AMIRADŽIBI from meat extracts. It is a base which is readily soluble in water, crystallizing in flat needles. It is precipitated by phosphotungstic acid and by silver nitrate in the presence of an excess of barium hydrate, and forms a copper compound which crystallizes in hexagonal plates. Carnosine, which also occurs, according to KRIMBERG, in fresh meat to the extent of 1.3 p. m., is probably a histidine derivative (GULEWITSCH) which is identical with *ignotine*, isolated by KUTSCHER from meat extract. According to KUTSCHER [1] these extractive bodies are more likely isomeric bodies.

Carnitine, $C_7H_{15}NO_3$, is another base isolated by GULEWITSCH and KRIMBERG from meat extracts, having a strong alkaline reaction, and is very readily soluble in water, and which KRIMBERG also found in fresh meat. Carnitine according to KRIMBERG [2] is a trimethylamine derivative and probably trimethyloxybutyrobetaine with the formula $(CH_3)_3N\left\langle\begin{array}{l} O\text{———————}CO \\ \qquad\qquad\qquad\quad | \\ CH_2-CH.OH-CH_2 \end{array}\right.$. According to him it is also very probably identical with *novaine* prepared by KUTSCHER from meat extracts, which is also a trimethylamine derivative. It gives crystalline double compounds with platinum, gold and mercuric chlorides, among which the following, $C_7H_{15}NO_3 2HgCl_2$, with a melting-point of 196–197° C., is especially used in the isolation of the base.

From LEIBIG's extract of beef KUTSCHER has isolated besides the above-mentioned *ignotine* and *novaine*, several other bodies, *neosine*, $C_6H_{17}NO_2$, which according to KUTSCHER and ACKERMANN is a homologue of choline, *vitiatine* (as gold salt, $C_5H_{14}N_6.2HCl.2AuCl_3$), *carnomuscarine*, *methylguanidine* (also found by GULEWITSCH), *oblitine*, $G_{18}H_{38}N_2O_5$, which probably contains two novaine groups, which corresponds well with KRIMBERG's [3] view, and also *choline* and *neurinė*. ZUNZ [4] has been able to obtain from fresh meat the three so-called hexone

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[1] Gulewitsch and Amiradžibi, Zeitschr. f. physiol. Chem., **30**; Gulewitsch, *ibid.*, **50**, **51**, and **52**; Krimberg, *ibid.*, **48**; Kutscher, *ibid.*, **50** and **51**.

[2] Gulewitsch and Krimberg, Zeitschr. f. physiol. Chem., **45**; Krimberg, *ibid.*, **49**, **50**, **53**, **56**.

[3] Kutscher, Zeitschr. f. Unters. d. Nahrungs- u. Genussmittel, **10**, **11**, Centralbl. f. Physiol., **19** and **21**, Zeitschr. f. physiol. Chem., **48**, **49**, **50**, **51**, with Ackermann, *ibid.*, **56**; Gulewitsch, *ibid.*, **47**; Krimberg, *ibid.*, **56**.

[4] Zunz, reference in Centralbl. f. Physiol., **18**, 852.

bases besides leucine, aspartic and glutamic acids, and MICKO[1] found in meat extracts small quantities of alanine, glutamic acid, taurine and inosite, but no dipeptides. In fish-flesh SUWA[2] found creatine, creatinine and methylguanidine, but not the numerous bodies found by KUTSCHER in meat extract. In crab extract KUTSCHER and ACKERMANN[3] found no creatine and creatinine, but among others betaine and two new bases, *crangitine*, $C_{13}H_{20}N_2O_4$ and *crangonine*, $C_{13}H_{26}N_2O_3$.

The base *musculamine*, isolated by ETARD and VILA on the hydrolysis of veal, is nothing but cadaverine, according to POSTERNAK.[4]

Inosinic acid has been discussed on page 179. We must also include among the nitrogenous extractives those bodies which were first discovered by GAUTIER[5] and which occur only in very small quantities, namely, the leucomaines, *xanthocreatinine*, $C_5H_{10}H_4O$, *crusocreatinine*, $C_5H_8N_4O$, *amphicreatine*, $C_9H_{19}N_7O_4$, and *pseudoxanthine*, $C_4H_5N_5O$.

In the analysis of meat, and for the detection and separation of the various extractive bodies of meat, we make use of the systematic method as suggested by GAUTIER,[6] for details of which the reader is referred to the original article.

Phosphocarnic acid[7] is a complicated substance, first isolated by SIEGFRIED from meat extracts, which yields as cleavage products succinic acid, paralactic acid, carbon dioxide, phosphoric acid, and a carbohydrate group, besides the previously mentioned carnic acid, which is identical with or nearly related to antipeptone. It stands, according to SIEGFRIED, in close relation to the nucleins, and as it yields peptone (carnic acid), it is designated as a *nucleon* by SIEGFRIED. Phosphocarnic acid may be precipitated as an iron compound, *carniferrine*, from the extract of the muscles free from proteins. The quantity of phosphocarnic acid, calculated as carnic acid, can be determined by multiplying the quantity of nitrogen in the compound by the factor 6.1237 (BALKE and IDE). In this way SIEGFRIED found 0.57–2.4 p. m. carnic acid in the resting muscles of the dog, and M. MÜLLER 1–2 p. m. in the muscles of adults and a maximum of 0.57 p. m. in those of new-born infants. According to CAVAZZANI nucleon occurs to a much greater extent in oysters, namely, an average of 3.725 p. m. It also occurs, as he and MANICARDI found, in the plant kingdom. Phosphocarnic acid has not been prepared in the pure state and possesses on this account a variable composition; according to SIEGFRIED it serves as a source of energy in the muscles and is consumed during work. Besides, by means of its property of forming soluble salts with the alkaline earths, as also an iron combination soluble in alkalies, it acts as a means of transportation for these bodies in the animal body.

Phosphocarnic acid is prepared from the extract free from protein by first removing the phosphate by $CaCl_2$ and NH_3. The acid is precipitated as carniferrine by ferric chloride from the filtrate while boiling.

The non-nitrogeneous extractive bodies of the muscles are *inosite, glycogen, sugar,* and *lactic acid.*

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[1] Zeitschr. f. physiol. Chem., **56**.

[2] Centralbl. f. Physiol., **22**, 307.

[3] Zeitschr. f. Unters. d. Nahrungs-u. Genussmittel, **13** and **14**.

[4] Etard and Vila, Compt. rend., **135**; Posternak, *ibid.*, **135**.

[5] See Maly's Jahresber., **16**, 523.

[6] *Ibid.*, **22**, 335.

[7] In regard to carnic acid and phosphocarnic acid, see the works of Siegfried, Arch. f. (Anat. u.) Physiol., 1894, Ber. d. deutsch. chem. Gesellsch., **28**, and Zeitschr. f. physiol. Chem., **21** and **28**; M. Müller, *ibid.*, **22**; Krüger, *ibid.*, **22** and **28**; Balke and Ide, *ibid.*, **21**, and Balke, *ibid.*, **22**; Macleod, *ibid.*, **28**; E. Cavazzani, Centralbl. f. Physiol., **18**, 666; Panella, Maly's Jahresber., **34**.

Inosite, $C_6H_{12}O_6+H_2O=C_6H_6(OH)_6+H_2O$. This body, discovered by SCHERER, is not a carbohydrate, but belongs to the hydroaromatic compounds, and is a hexahydroxybenzene (MAQUENNE [1]). That it stands in certain relation to the carbohydrates follows from the fact that NEUBERG obtained some furfurol from inosite by distillation with phosphoric anhydride and also that P. MEYER [2] found fermentation lactic acid in the urine of rabbits after the introduction of inosite *per os*. It has been known for some time that inosite undergoes lactic acid fermentation. The acid formed thereby is sarcolactic acid according to HILGER and fermentation lactic acid according to VOHL.[3]

Inosite is found in the muscles, liver, spleen, leucocytes, kidneys, suprarenal capsule, lungs, brain, testicles, and in the urine in pathological cases, and as traces in normal urine. ROSENBERGER attempted to show that in certain animals (rabbits) and organs (muscles) the inosite did not occur free, but as an inositogen, but this claim has not sufficient foundation and it is denied by STARKENSTEIN.[4] It is found very widely distributed in the vegetable kingdom, especially in the unripe fruit of green beans (Phaseolus vulgaris), and therefore it is also called PHASEOMANNITE. According to WINTERSTEIN a phosphorized compound occurs in the vegetable kingdom which yields inosite as a cleavage product and whose Mg and Ca compound is called *phytin*. POSTERNAK considers this body as an anhydroxymethylenediphosphoric acid. From the cleavage experiments of WINTERSTEIN as well as the observations of SUZUKI, YOSHIMURA and TAKAISHI [5] on the occurrence in rice-bran of a special enzyme, *phytase*, which splits phytin into inosite and phosphoric acid, it seems as if this body is more likely an inosite-phosphoric acid. Inosite is found in plants, especially in the developing organs (MEILLÈRE), and according to STARKENSTEIN [6] it occurs to a greater extent in the organs of young animals as compared with those of older animals. From this it follows that inosite is probably not a decomposition product of metabolism, but rather a body necessary for the development of the cells.

Inosite, which nearly without exception is inactive mesoinosite, crystallizes in large, colorless, rhombic crystals of the monoclinic system, or, if not pure and if only a small quantity crystallizes, it forms

[1] Bull. soc. chem. (2), **47** and **48**; Compt. rend., **104**.

[2] Neuberg, Bioch. Zeitschr., **9**; P. Mayer, *ibid.*, **9**.

[3] Hilger, Annal. d. Chem. u. Pharm., **160**; Vohl, Ber. d. d. Chem. Gesellsch., **9**.

[4] Rosenberger, Zeitschr. f. physiol. Chem., **56**, **57**, and **58**; Starkenstein, *ibid.*, **58**.

[5] Winterstein, Ber. d. d. chem. Gesellsch., **30**, and Zeitschr. f. physiol. chem., **58**; Posternak, Contribution a l'étude chim. de l'assimilation chlorophyllienne. Revue générale botanique, Tome **12** (1900), and Compt. rend., **137**; Suzuki, Yoshimura and Takaishi, Bull. agric. Univers. Tokio, **7**.

[6] Meillère Jour. d. Chim. et Pharm. (6) 28; Starkenstein, Zeitschr. f. Exp. Path. u. Therap., **5**.

groups of fine crystals similar to cauliflower. It loses its water of crystallization at 110° C., also if exposed to the air for a long time. Such exposed crystals are non-transparent and milk-white. The crystals melt at 225° C. when dry. Inosite dissolves in 7.5 parts of water at ordinary temperature, and the solution has a sweetish taste. It is insoluble in strong alcohol and in ether. It dissolves cupric hydrate in alkaline solutions, but does not reduce on boiling. It gives negative results with MOORE'S test and with BÖTTGER-ALMÉN'S bismuth test. It does not ferment with beer-yeast, but may undergo lactic- and butyric-acid fermentation. With an excess of nitric acid inosite is oxidized to *rhodizonic acid*, and the following reaction depends upon this.

If inosite is evaporated to dryness on platinum-foil with nitric acid and the residue treated with ammonia and a drop of calcium chloride solution and carefully re-evaporated to dryness, a beautiful rose-red residue is obtained (SHERER'S inosite test). If we evaporate an inosite solution to incipient dryness and moisten the residue with a little mercuric nitrate solution, there is obtained a yellowish residue on drying, which becomes a beautiful red on strongly heating. The coloration disappears on cooling, but it reappears on gently warming (GALLOIS' inosite test). New inosite reactions have been suggested by DENIGÈS.[1]

To prepare inosite from a liquid or from a watery extract of a tissue, the proteins are first removed by coagulation at boiling heat. The filtrate is precipitated by sugar of lead, this filtrate boiled with basic lead acetate and allowed to stand 24–48 hours. The precipitate thus obtained, which contains all the inosite, is decomposed in water by H_2S. The filtrate is strongly concentrated, treated with 2–4 vols. hot alcohol, and the liquid removed as soon as possible from the tough or flaky masses which ordinarily separate. If no crystals separate from the liquid within twenty-four hours, then treat with ether until the liquid has a milky appearance and allow it to stand. In the presence of a sufficient quantity of ether, crystals of inosite separate within twenty-four hours. The crystals thus obtained, as also those which are obtained from the alcoholic solution directly, are recrystallized by redissolving in very little boiling water and adding 2–4 vols. of alcohol. MEILLÈRE [2] and others have suggested modifications in the methods for detecting and quantitatively estimating inosite.

Scyllite is a body which is isomeric with inosite, according to JOH. MÜLLER,[3] and which was found long ago in the kidneys, liver and spleen of Plagiostomata. Scyllite crystallizes in shining prisms, is soluble in water 1 : 100 at 180° C., is similar to inosite in its reactions, but has a much higher melting-point, namely about 360° C.

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[1] Compt. rend. soc. biol., 62.

[2] Compt. rend. soc. biol., 60, and Journ. d. Chim. et Pharm., (6) 24; see also Starkenstein, Zeitschr. f. exp. Path. u. Ther., 5.

[3] Ber. d. d. chem. Gesellsch., 40.

Glycogen is a constant constituent of the living muscle, while it may be absent in the dead muscle. The quantity of glycogen varies in the different muscles of the same animal. BÖHM found 10 p. m. glycogen in the muscles of cats, and moreover he found a smaller amount in the muscles of the extremities than in those of the rump. MOSCATI found an average of 4 p. m. in human muscles, and SCHÖNDORFF [1] has found a maximum of 37.2 p. m. in the dog-muscle. Reports as to the quantity of glycogen in the heart are conflicting; although the heart is considered as somewhat poorer in glycogen than the other muscles, still this difference is not very great, and can be explained by the ready disappearance of glycogen from the heart after death, as well as after starvation and after strong work (BORUTTAU, JENSEN[2]). Work and food have a great influence upon the quantity of glycogen. BÖHM found 1–4 p. m. glycogen in the muscles of fasting animals, and 7–10 p.m. after partaking of food. As stated in Chapter VIII, work, starvation, and lack of carbohydrates in the food cause the glycogen to disappear, earlier, from the liver than from the muscles.

The *sugar of the muscles,* of which only traces occur in the living muscle, and which is probably formed after the death of the muscle from the muscle-glycogen, is, according to the investigations of PANORMOFF, in part dextrose, but consists chiefly of maltose (OSBORNE and ZOBEL [3]) with some dextrin.

Lactic Acids. Of the oxypropionic acids with the formula $C_3H_6O_3$ there is one, ethylene lactic acid, $CH_2(OH).CH_2.COOH$, which is not found in the animal body, and therefore has no physiological chemical interest. Indeed only α-oxypropionic acid or ethylidene lactic acid, $\begin{matrix}CH_3\\ CH(OH)\\ COOH\end{matrix}$, of which there are three physical isomers, is of importance. These three ethylidene lactic acids are the ordinary, optically inactive FERMENTATION LACTIC ACID, the dextrorotatory PARALACTIC OR SARCOLACTIC ACID, and the LEVOLACTIC ACID obtained by SCHARDINGER by the fermentation of cane-sugar by means of a special bacillus. This levolactic acid, which has also been detected by BLACHSTEIN in the culture of GAFFKY'S typhoid bacillus in a solution of sugar and peptone, and which is formed by various vibriones, need not be described here.[4]

[1] Böhm, Pflüger's Arch., **23**, 44; Schöndorff, *ibid.*, **99**; Moscati, Hofmeister's Beiträge, **10**.

[2] Boruttau, Zeitschr. f. physiol. Chem., **18**; Jensen, *ibid.*, **35**.

[3] Panormoff, Zeitschr. f. physiol. Chem., **17**; Osborne and Zobel, Journ. of Physiol., **29**.

[4] See Schardinger, Monatshefte f. Chem., **11**; Blachstein, Arch. des sciences biol. de St. Pétersbourg, **1**, 199; Kuprianow, Arch. f. Hygiene, **19**, and Gosio, *ibid.*, **21**; Herzog and Hörth, Zeitschr. f. physiol. Chem., **60**.

The *fermentation lactic acid*, which is formed from lactose by allowing milk to sour, and by the acid fermentation of other carbohydrates, is considered to exist in small quantities in the muscles (Heintz), in the gray matter of the brain (Gscheidlen), and in diabetic urine. The occurrence of fermentation lactic acid in the brain and other organs has recently been disputed by Moriya.[1] During digestion this acid is also found in the contents of the stomach and intestine, and as alkali lactate in the chyle. The *paralactic acid* is, at all events, the true acid of meat extracts, and this alone has been found with certainty in dead muscle. The lactic acid which is found in the brain, spleen, lymphatic glands, thymus, thyroid gland, blood, bile, pathological transudates, osteomalacious bones, in perspiration in puerperal fever, in the urine after fatiguing marches, in acute yellow atrophy of the liver, in poisoning by phosphorus, and especially after extirpation of the liver seems to be paralactic acid..

The origin of paralactic acid in the animal organism has been sought by several investigators, who took for basis the researches of Gaglio, Minkowski, and Araki, in a decomposition of protein in the tissues. Gaglio claims a lactic-acid formation by passing blood through the surviving kidneys and lungs. He also found 0.3–0.5 p. m. lactic acid in the blood of a dog after protein food, and only 0.17–0.21 p. m. after fasting for forty-eight hours. According to Minkowski the quantity of lactic acid eliminated by the urine in animals with extirpated livers is increased with protein food, while the administration of carbohydrates has no effect. Araki has also shown that if we produce a scarcity of oxygen in animals (dogs, rabbits, and hens) by poisoning with carbon monoxide, by the inhalation of air deficient in oxygen, or by any other means, a considerable elimination of lactic acid (besides dextrose and also often albumin) takes place through the urine, an observation which has been confirmed by Saito and Katsuyama.[2] As a scarcity of oxygen, according to the ordinary statements, produces an increase of the protein catabolism in the body, the increased elimination of lactic acid in these cases must be due in part to an increased protein destruction and in part to a diminished oxidation.

Araki has not drawn such a conclusion from his experiments, but he considers the abundant formation of lactic acid to be due to a cleavage of the sugar formed from the glycogen. He found that in all cases where lactic acid and sugar appeared in the urine the quantity of glycogen in

[1] Heintz, Annal. d. Chem. u. Pharm., **157**, and Gscheidlen, Pflüger's Arch., 8, 171; Moriya, Zeitschrift f. physiol. Chem., **43**.

[2] Gaglio, Arch. f. (Anat. u.) Physiol., 1886; Minkowski, Arch. exp. Path. u. Pharm., **21** and **31**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**, and **19**; Saito and Katsuyama, *ibid.*, **32**.

the liver and muscles was always diminished. He also calls attention to the fact that dextrolactic acid may be formed from glycogen, as directly observed by EKUNINA,[1] and also to the numerous observations on the formation of lactic acid and the consumption of glycogen in muscular activity. Without denying the possibility of a formation of lactic acid from protein, he states that with lack of oxygen we have to deal with an incomplete combustion of the lactic acid derived by a cleavage of the sugar. HOPPE-SEYLER[2] also positively defends the view as to the formation of lactic acid from carbohydrates. He was of the opinion that lactic acid is produced from the carbohydrates by the cleavage of the sugar only with lack of oxygen, while with sufficient oxygen the sugar is burned into carbon dioxide and water. The formation of lactic acid in the absence of free oxygen and in the presence of glycogen or dextrose is, according to HOPPE–SEYLER, very probably a function of all living protoplasm. In the anaerobic metabolism of the animal cells, according to the recent investigations on alcoholic fermentation in the tissues (see Chapter VIII), carbon dioxide and alcohol are formed from the sugar, with lactic acid as an intermediary step; but even if this view be correct and when the cells, as STOKLASA[3] and his collaborators have shown, contain a lactic-acid-forming enzyme, it is not known what kind of lactic acid is here produced. MORISHIMA believes that an increase in the lactic acid in the liver occurs after death, probably from the liver glycogen, but this acid is chiefly fermentation lactic acid, and the fact must not be overlooked that INOUYE and KONDO[4] found dextrorotatory lactic acid on the autolysis of muscles.

ASHER and JACKSON[5] experimented by transfusing blood (with and without the addition of sugar) through the lower extremities of dogs, and neither in these experiments nor in those where the larger organs (liver and abdominal viscera) were excluded from the circulation could they detect any increase of lactic acid due to the sugar. Although these last-mentioned investigations do not show any formation of lactic acid from carbohydrates, still, on the other hand, we have recent investigations that make such an origin for lactic acid very probable. Thus EMBDEN[6] found, on percolating blood through a surviving liver rich in glycogen, that lactic acid was formed, and also that this acid was produced in abundance when blood rich in sugar was transfused

[1] Journ. f. prakt. Chem. (N. F.), **21**.

[2] Virchow's Festschrift, also Ber. d. deutsch. chem. Gesellsch., **25**, Referatb., 685.

[3] Simácek, Centralbl. f. Physiol., **17**; Stoklasa, Jelinek, and Cerny, *ibid.*, **16**.

[4] Morishima, Arch. f. exp. Path. u. Pharm., **43**; Inouye and Kondo, Zeitschr. f. Physiol. Chem., **54**.

[5] Zeitschr. f. Biologie, **41**.

[6] Centralbl. f. Physiol., **18**, 832.

through a glycogen-free liver, while, on the contrary, blood poor in sugar led to only a very inconsiderable formation of lactic acid. The investigations of A. R. MANDEL and LUSK[1] also indicate a formation of lactic acid from carbohydrates, in the animal body. They have shown that in dogs, after phosphorus poisoning, an abundance of lactic acid occurs in the blood and urine, and that this disappears from these fluids on bringing about a phlorhizin diabetes in the animal. Phosphorus intoxication caused no lactic-acid formation in a phlorhizin-diabetic dog. Although it is difficult to give a satisfactory explanation of the results of these experiments, still it seems probable that by elimination of the sugar in phlorhizin diabetes a mother-substance of the lactic acid is lost.

The carbohydrates, as well as the proteins, it seems, must be considered as the material from which the lactic acid is formed in the body. In a previous chapter (VIII) we mentioned the formation of lactic acid in the animal body by a deamination of alanine, and this gives us an indication of a lactic-acid formation from protein. Phosphocarnic acid is considered by SIEGFRIED as another source of sarcolactic acid.

The lactic acids are amorphous. They have the appearance of colorless or faintly yellowish, acid-reacting syrups which mix in all proportions with water, alcohol, or ether. The salts are soluble in water, and most of them also in alcohol. The two acids are differentiated from each other by their different optical properties—paralactic acid being dextrogyrate, while fermentation lactic acid is optically inactive—also by their different solubilities and the different amounts of water of crystallization of the calcium and zinc salts. The zinc salt of fermentation lactic acid dissolves in 58–63 parts of water at 14–15° C., and contains 18.18 per cent water of crystallization, corresponding to the formula $Zn(C_3H_5O_3)_2 + 3H_2O$. The zinc salt of paralatic acid dissolves in 17.5 parts of water at the above temperature and contains ordinarily 12.9 per cent water, corresponding to the formula $Zn(C_3H_5O_3)_2 + 2H_2O$. The calcium salt of fermentation lactic acid dissolves in 9.5 parts water and contains 29.22 per cent (=5 molecules) water of crystallization, while calcium paralactate dissolves in 12.4 parts water and contains 24.83 or 26.21 per cent (=4 or $4\frac{1}{2}$ molecules) water of crystallization. Both calcium salts crystallize, not unlike tyrosine, in spears or tufts of very fine microscopic needles. HOPPE-SEYLER and ARAKI, who have closely studied the optical properties of the lactic acids and lactates, consider the lithium salt as best suited for the preparation and quantitative estimation of the lactic acids. The lithium salt contains 7.29 per cent Li. For further information as to the salts and specific rotation of the lactic acids see HOPPE-SEYLER-THIERFELDER'S Handbuch, 8. Aufl., 1909.[2]

[1] Amer. Journ. of Physiol., 16.

[2] See also E. Jungfleisch, Compt. rend., **139**, **140**, and **142**.

Lactic acids may be detected in organs and tissues in the following manner: After complete extraction with water, the protein is removed by coagulation at boiling temperature and the addition of a small quantity of sulphuric acid. The liquid is then exactly neutralized, while boiling, with caustic baryta, and then evaporated to a syrup after filtration. The residue is precipitated with absolute alcohol, and the precipitate completely extracted with alcohol. The alcohol is entirely distilled from the united alcoholic extracts, and the neutral residue is shaken with ether to remove the fat. The residue is dissolved in water and phosphoric acid added, and the solution repeatedly shaken with fresh quantities of ether, which dissolves the lactic acid. The ether is now distilled from the united ethereal extracts, the residue dissolved in water, and this solution carefully warmed on the water-bath to remove the last traces of ether and volatile acids. A solution of zinc lactate is prepared from this filtered solution by boiling with zinc carbonate, and this is evaporated until crystallization commences, and is then allowed to stand over sulphuric acid. An analysis of the salts is necessary in careful work. In regard to methods for the detection and quantitative estimation of lactic acid we must refer to larger hand-books and to the work of JERUSALEM.[1]

Fat is never absent in the muscles. Some fat is always found in the intermuscular connective tissue; but the muscle-fibres themselves also contain fat. The quantity of fat in the real muscle substance is always small, usually amounting to about 10 p. m. or somewhat more. A considerable quantity of fat in the muscle-fibres is found only in fatty degeneration. A part of the muscle-fat can be readily extracted, while another part can be extracted only with the greatest difficulty. This latter part, it is claimed, exists finely divided in the contractile substance itself and is richer in free fatty acids, standing, according to ZUNTZ and BOGDANOW,[2] in close relation to the activity of the muscles because it is consumed during work. *Lecithin* is a regular constituent of the muscles, and it is quite possible that the fat which is difficult of extraction and which is rich in fatty acids depends in part on a decomposition of the lecithin and the phosphatides. ERLANDSEN has shown, that *phosphatides* of various kinds occur in the muscles and indeed different quantities in different muscles. According to him the ox-heart muscle is richer in phosphatides than the muscle of the thigh, and RUBOW[3] claims that the heart of the dog is richer in phosphatides than the striated muscle. ERLANDSEN found lecithin and diamino-phosphatide in the heart as well as the thigh-muscle, while the monoamido-phosphatide cuorin, which occurs abundantly in the heart, is found as traces in the thigh-muscle.

[1] Bioch. Zeitschr., **12**.

[2] Arch. f. (Anat. u.) Physiol., 1897. *è*

[3] Erlandsen, Zeitschr. f. physiol. Chem **51**; Rubow, Arch. f. exp. Path. u. Pharm., **52**.

The Mineral Bodies of the Muscles. The ash remaining after burning the muscle, which amounts to about 10–15 p. m., calculated on the moist muscle, is acid in reaction. The largest constituent of the ash is potassium, whose occurrence, according to MACALLUM, is restricted to the dark diagonal bundles, and phosphoric acid. Next in amount we have sodium and magnesium, and lastly calcium, chlorine, and iron oxide. Sulphates exist only as traces in the muscles, but are formed by the burning of the proteins of the muscles, and therefore occur in abundant quantities in the ash. The muscles contain such a large quantity of potassium and phosphoric acid that potassium phosphate seems to be, unquestionably, the predominating salt. Chlorine is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is, as a rule, considerably greater than that of calcium. Iron occurs only in very small amounts. SCHMEY [1] found variations between 0.0129 p. m. (rabbits) and 0.0793 p. m. (human), calculated on the fresh muscle substance. The heart-muscle was comparatively richer in iron, 0.06–0.109 p. m.

URANO [2] has removed the salts of the intermediary fluid (blood, lymph) from frogs' muscle by treating them with an isotonic cane-sugar solution (of 6 per cent) and in this manner found that the sodium did not belong to the muscle substance itself, but to the intermediary fluid, while at least a small part of the chlorine is a true muscle constituent. He also calculated from the quantity of sodium that the intermediary fluid, if it has about the same composition as the muscle plasma, makes up about one-sixth of the volume of the muscle. According to more recent investigations of URANO the possibility of a disturbance in the osmotic properties of the muscle fibres by the sugar solution is not entirely excluded, and the question whether the muscle fibres are free from sodium or not has therefore not been positively decided. FAHR'S [3] researches make the absence of sodium in frog's muscle very probable.

The importance of the various mineral bodies for the function of the muscles has been studied by several experimenters (LOEB, LINGLE, HOWELL, OVERTON, LANGENDORFF and HUECK, and others [4]). Further proof as to the previously discussed ion action of the electroytes and the antagonism of various ions has been given by many very interesting investigations. These researches also indicate that each of the ions Na, Ca, and K plays a certain part in the maintenance of the excitability,

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[1] Macallum, Journ. of Physiol., **32**; Schmey, Zeitschr. f. physiol. Chem., **39**.

[2] Zeitschr. f. Biol., **50**.

[3] Urano, *ibid.*, **51**; Fahr, *ibid.*, **52**.

[4] Loeb, Amer. Journ. of Physiol., **3**, and Pflüger's Arch., **80**, **91**; Lingle, Amer. Journ. of Physiol., **4** (also references to literature); Overton, Pflüger's Arch., **92** and **105**; Langendorff and Hueck, *ibid.*, **96**.

in the contraction and in the fatigue of the muscle (heart); still these investigations have not led to concordant results, so that we are not yet clear as to the action of these ions. Nevertheless it seems to be established that the combined action of various ions is a necessity for the normal function of the muscles. It has also been shown that it is possible to maintain the muscle (the heart) in regular activity for a long time by means of a transfusion of liquid saturated with oxygen and which contained about 7 p. m. NaCl, besides small amounts of $CaCl_2$ (0.2 p. m.), KCl (0.1 p. m.), and $NaHCO_3$ (0.1 p. m.).

The *gases* of the muscles consist of large quantities of carbon dioxide besides traces of nitrogen.

In regard to the permeability of the muscles for various bodies there are the complete investigations of Overton.[1] The different sheaths of the muscles, the sarcolemma and perimysium internum, offer no very great resistance to the diffusion of the most soluble crystalloid compounds, while the muscle-fibres, on the contrary (exclusive of the sarcolemma), are almost if not entirely impervious to most inorganic compounds and to many organic compounds. The muscle-fibres themselves are actually semipermeable structures which are permeable to water but not to the molecules or ions of sodium chloride and of potassium phosphate. The muscle-fibres, as well as the various sheaths, are impermeable to colloids.

The behavior of the numerous bodies investigated cannot be discussed in this work. The general rule is as follows: All compounds which, besides having a marked solubility in water, are readily soluble in ethyl ether, in the higher alcohols, in olive-oil and in similar organic solvents, or are not much less soluble in the last-mentioned solvents than in water, pass through the living muscle-fibres with great ease. The greater the difference between the solubility of a compound in water and in the other solvents mentioned, the slower does the passage into the muscle-fibres take place. The permeability changes essentially on the death of the muscle.

The living muscle-fibres are readily permeable to oxygen, carbon dioxide, and ammonia, while the hexoses and disaccharides do not readily pass into them. It is very remarkable that a great portion of those compounds which take part in the normal metabolism of plants and animals belong to those bodies to which the muscle-fibres (and also other cells) are entirely or at least nearly impermeable. On the contrary, derivatives can be prepared from these bodies which pass into the cells very readily, and Overton finds that it is not impossible that the organism

[1] Pflüger's Arch., **92**. See also Höber, *ibid.*, **106**, and Hamburger, Osmotischer Druck und Ionenlehre, Bd. **3**.

in part makes use of a similar artifice in order to regulate the concentration of the nutritive bodies within the protoplasm.

Rigor Mortis of the Muscles. If the influence of the circulating oxygenated blood is removed from the muscles, as after the death of the animal or by ligature of the aorta or the muscle-arteries (STENSON'S test), *rigor mortis* sooner or later takes place. The ordinary rigor appearing under these circumstances is called the spontaneous or the fermentative rigor, because it seems to depend in part on the action of an enzyme. A muscle may also become stiff for other reasons. The muscles may become momentarily stiff by warming, in the case of frogs to 40°, in mammalia to 48–50°, and in birds to 53° C. The heat-rigor depends upon the coagulation of certain proteins, and its occurrence at lower temperatures in cold-blooded as compared with warm-blooded animals is due, according to v. FÜRTH, to the fact that in the first a soluble myogen fibrin occurs preformed in the muscle which coagulates at 30–40° C., while in the warm-blooded animals the coagulating substance is musculin (myosin of v. FÜRTH) which coagulates at a higher temperature. According to INAGAKI [1] the various stages in contractions occurring on heating a muscle (frog) do not correspond to those of the coagulation of the protein which would occur on heating the muscle plasma, and this probably depends upon the fact that the reaction of the muscle changes on heating with the formation of lactic acid. Distilled water may also produce a rigor in the muscles (water-rigor). Acids, even very weak ones, such as carbon dioxide, may quickly produce a rigor (acid-rigor), or hasten its appearance. A number of chemically different substances, such as chloroform, ether, alcohol, ethereal oils, caffeine, and many alkaloids, produce a similar effect. The rigor which is produced by means of acids or other agents which, like alcohol, coagulate proteins must be considered as produced by entirely different processes from those causing spontaneous rigor.

When the muscle passes into rigor mortis it becomes shorter and thicker, harder and non-transparent, and less ductile. The acid part of the amphoteric reaction becomes stronger, which is explained by most investigators by the assumption of a formation of lactic acid. There is hardly any doubt that this increase in acidity may at least in part be due to a transformation of a part of the diphosphate into monophosphate by the lactic acid. The reports in regard to the presence or absence of free lactic acid in the rigor-mortis muscle are contradictory.[2] Besides

[1] Zeitschr. f. Biol., 48. See also E. B. Meigs, Amer. Journ. of Physiol., 24.

[2] It is impossible to enter into the details of the disputed theories as to the reaction of the muscles, etc. We shall only refer to the works of Röhmann, Pflüger's Arch., 50 and 55, and Heffter, Arch. f. exp. Path. u. Pharm., 31 and 38

the formation of acid, the chemical processes which take place in rigor of the muscles are the following: By the coagulation of the plasma a myosin-clot is produced which is the cause of the hardening and of the diminished transparency of the muscle; but this view must be changed on account of the researches of v. FÜRTH, which have shown that the clot consists of myogen fibrin and myosin fibrin. The appearance of this clot may be hastened by the simultaneous occurrence of lactic acid. Carbon dioxide is also formed, which does not seem to be a direct oxidation product, but a product of the cleavage processes. HERMANN [1] claims that carbon dioxide is produced in the removed muscle, even in the absence of oxygen, when it passes into rigor mortis. In connection with this view we must call attention to FOLIN'S [2] observations that no protein coagulation took place in rigor under special conditions.

As many investigators admit of an increased formation of lactic acid on the appearance of rigor mortis, the question arises, from what constituents of the muscle is this acid derived? The most probable explanation is that the lactic acid is produced from the glycogen, as certain investigators, such as NASSE and WERTHER, have observed a decrease in the quantity of glycogen in rigor of the muscle. On the other side, BÖHM [3] has observed cases in which no consumption of glycogen took place in rigor of the muscle, and he also found that the quantity of lactic acid produced is not proportional to the quantity of glycogen. It is therefore possible that the consumption of glycogen and the formation of lactic acid in the muscles are two processes independent of each other, and, as above stated in regard to the formation of paralactic acid, the lactic acid of the muscle may be considered as a decomposition product of protein. The origin of the carbon dioxide is also not to be sought for in the decomposition of the glycogen or dextrose. PFLÜGER and STINTZING [4] found that in the muscle a substance occurs which evolves large quantities of carbon dioxide on boiling with water, and it is probably this substance which is decomposed with the formation of carbon dioxide in tetanus as well as in rigor. In this connection we call attention to the fact that phosphocarnic acid yields lactic acid as well as carbon dioxide as cleavage products.

After the muscles have been rigid for some time they relax again and

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These works contain also the researches of the earlier investigators more or less completely.

[1] Untersuchungen über den Stoffwechsel der Muskeln, etc., Berlin, 1867.

[2] Amer. Journ. of Physiol., **9**.

[3] Nasse, Beitr. z. Physiol. der kontrakt. Substanz, Pflüger's Arch., **2**; Werther, *ibid.*, **46**; Böhm, *ibid.*, **23** and **46**; Moscati, Hofmeister's Beiträge, **10**.

[4] Pflüger's Arch., **18**.

become softer. This is in part produced by the strong acid dissolving the myosin-clot and in part by autolytic processes (Vogel).[1]

Metabolism in the Inactive and Active Muscles. It is admitted by a number of prominent investigators, Pflüger and Colasanti, Zuntz and Röhrig,[2] and others, that the metabolism in the muscles is regulated by the nervous system. When at rest, when there is no mechanical exertion, there exists a condition which Zuntz and Röhrig have designated "*chemical tonus.*" This tonus seems to be a reflex tonus, for it may be reduced by discontinuing the connection between the muscles and the central organ of the nervous system by cutting through the spinal cord or the muscle-nerves. The possibility of reducing the chemical tonus of the muscles in various ways offers an important means of deciding the extent and kind of chemical processes going on in the muscles when at rest. In comparative chemical investigation of the processes in the active and the inactive muscles several methods of procedure have been adopted. The same active and inactive muscles have been compared after removal, also the arterial and venous muscle-blood in rest and activity, and lastly the total exchange of material, the receipts and expenditures of the organism, have been investigated under these two conditions.

By investigations according to these several methods it was found that the resting muscle takes up oxygen from the blood and returns to it carbon dioxide, and also that the quantity of oxygen taken up is greater than the oxygen contained in the carbon dioxide eliminated at the same time. The muscle, therefore, holds in some form of combination a part of the oxygen taken up while at rest. During activity the exchange of material in the muscle, and therewith the exchange of gas, is increased. The animal organism takes up much more oxygen in activity than when at rest, and eliminates also considerably more carbon dioxide. The quantity of oxygen which leaves the body as carbon dioxide during activity is much larger than the quantity of oxygen taken up at the same time; and the venous muscle-blood is poorer in oxygen and richer in carbon dioxide during activity than during rest. The exchange of gases in the muscles during activity is the reverse of that at rest, for the active muscle gives up a quantity of carbon dioxide which does not correspond to the quantity of oxygen taken up, but is considerably greater. It follows from this that in muscular activity not only does oxidation take place, but also splitting processes occur. This also results from the fact that removed blood-free muscles when placed in an atmosphere devoid of

[1] R. Vogel, Unters. über Muskelsaft, Deutsch. Arch. f. klin. Med., 1902.

[2] See the works of Pflüger and his pupils in Pflüger's Arch., **4**, **12**, **14**, **16**, and **18**; Röhrig, *ibid.*, **4**. See also Zuntz, *ibid.*, **12**. In regard to the metabolism after curare poisoning, see also Frank and Voit, Zeitschr. f. Biologie, **42**, and Frank and Gebhard, *ibid.*, **43**.

oxygen can labor for some time and still yield carbon dioxide (HERMANN[1]).

During muscular inactivity, in the ordinary sense, a consumption of glycogen takes place. This is inferred from the observations of several investigators that the quantity of glycogen is increased and its corresponding consumption reduced in those muscles whose chemical tonus is reduced either by cutting through the nerve or for other reasons (BERNARD, CHANDELON, VAY,[2] and others). In activity this consumption of glycogen is increased, and it has been positively proven by the researches of several investigators (NASSE, WEISS, KÜLZ, MARCUSE, MANCHÉ, MORAT and DUFOUR[3]) that the quantity of glycogen in the muscles in activity decreases quickly and freely. As shown by the researches of CHAUVEAU and KAUFMANN, QUINQUAUD, MORAT and DUFOUR, CAVAZZANI, and especially those of SEEGEN,[4] the sugar is removed from the blood and consumed during activity. The recent investigations of JOH. MÜLLER, LOCKE and ROSENHEIM and CAMIS[5] have given direct proof of the consumption of sugar during muscular activity. In experiments on surviving hearts of different animals through which was perfused a salt solution containing sugar, they could detect an undoubted consumption of sugar which was quite considerable and which to all appearances was used as material for muscle work.

The amphoteric reaction of the inactive muscles is changed during activity to an acid reaction (DU BOIS-REYMOND and others), and the acid reaction increases, to a certain point, with the work. The quickly contracting pale muscles produce, according to GLEISS,[6] more acid during activity than the more slowly contracting red muscles. The acid reaction appearing during activity was formerly considered to be due to the formation of lactic acid, a view which has been contradicted by ASTASCHEWSKY, PFLÜGER, and WARREN, who found less lactic acid in the tetanized muscle than when at rest. MONARI also found a decrease in the quantity of lactic acid during activity, and according to HEFFTER

[1] l. c. In regard to gas exchange in removed muscles, see aso J. Tissot, Arch. de Physiol. (5), **6** and **7**, and Compt. rend., **120**.

[2] Chandelon, Pflüger's Arch., **13**; Vay, Arch. f. exp. Path. u. Pharm., **34**, which also contains the pertinent literature.

[3] Nasse, Pflüger's Arch., **2**; Weiss, Wien. Sitzungsber., **64**; Külz, in Ludwig's Festschrift, Marburg, 1890; Marcuse, Pflüger's Arch., **39**; Manché, Zeitschr. f. Biologie, **25**; Morat and Dufour, Arch. de Physiol. (5), **4**.

[4] Chauveau and Kaufmann, Compt. rend., **103**, **104**, and **105**; Quinquaud, Maly's Jahresber., **16**; Morat and Dufour, l. c.; Cavazzani, Centralbl. f. Physiol., **8**; Seegen, "Die Zuckerbildung im Thierkörper," Berlin, 1890, Centralbl. f. Physiol., **8**, **9**, and **10**; Arch. f. (Anat. u.) Physiol., 1895 and 1896; Pflüger's Arch., **50**.

[5] Joh. Müller, Zeitschr. f. allgem. Physiol., **3**; Camis, *ibid.*, **8**; Locke and Rosenheim, Journ. of Physiol., **36**.

[6] Pflüger's Arch., **41**.

the quantity of lactic acid in the muscle is diminished in tetanus produced by poison. Contrary to the results of these investigations, MARCUSE and WERTHER have been able to prove the formation of lactic acid during activity; still the reports are discordant. Other observations indicate a formation of lactic acid during activity. Thus SPIRO found an increase in the quantity of lactic acid in the blood during work. COLASANTI and MOSCATELLI found small quantities of lactic acid in human urine after strenuous marches, and WERTHER observed an abundance of lactic acid in the urine of frogs after tetanization. According to HOPPE-SEYLER, on the contrary, in agreement with his view in regard to the formation of lactic acid, it is not produced regularly during work, but only when insufficient oxygen is supplied. ZILLESEN[1] has also found that on artificially cutting off the oxygen from the muscles during life more lactic acid was formed than under normal conditions.

It is evident that the experiments with the muscles *in situ*—in other words, with muscles through which blood is passing—cannot yield any conclusion to the above question, as the lactic acid formed during work may perhaps be removed by the blood. The following objections can be made against those experiments in which lactic acid has been found, after moderate work, in the blood or the urine, as also especially against the experiments with removed active muscles, namely, that in these cases the supply of oxygen to the muscles was not sufficient, and that the lactic acid formed thereby is not, in accordance with the views of HOPPE-SEYLER, a perfectly normal process. Of importance in considering the formation of lactic acid in the muscle and the conflicting opinions on this subject is the work of FLETCHER and HOPKINS.[2] They find that in the preparation of the muscle and its preparation for the investigation for lactic acid several sources of error are possible. Thus mechanical irritation such as warming or treating the muscle with alcohol, which is not ice-cold, brings about the formation of lactic acid. It was also shown that the absence of oxygen is favorable to the formation or accumulation of lactic acid, while abundant oxygen supply acts reversely. The question as to the formation of lactic acid under different physiological conditions requires further study.

According to SIEGFRIED the amount of phosphocarnic acid is diminished during activity. MACLEOD claims that this is true only for intense muscular activity, while with ordinary work the organic phosphorus

[1] Astaschewsky, Zeitschr. f. physiol. Chem., 4; Warren, Pflüger's Arch., 24; Monari, Maly's Jahresber., 19; Heffter, Arch. f. exp. Path. u. Pharm., 31; Marcuse, l. c.; Werther, Pflüger's Arch., 46; Spiro, Zeitschr. f. physiol. Chem., 1; Colasanti and Moscatelli, Maly's Jahresber., 17, 212; Hoppe-Seyler, l. c., and Zeitschr. f. physiol. Chem., 19; Zillesen, *ibid.*, 15.

[2] Journ. of Physiol., 35.

not present as nucleons is diminished and the quantity of phosphates is increased. This stands in accord with WEYL and ZEITLER'S [1] observations that the active muscle contains more phosphoric acid than the inactive muscle. As in the dead muscle, so in the active muscle, the somewhat stronger acid reaction is in part due to a greater quantity of monophosphate.

The amount of proteins in the removed muscles is, according to the earlier investigators, decreased by work. The correctness of this statement is, however, disputed by other investigators. Earlier reports in regard to the nitrogenous extractive bodies of the muscle in rest and in activity are likewise uncertain. According to the recent researches of MONARI [2] the total quantity of creatine and creatinine is increased by work, and indeed the amount of creatinine is especially augmented by an excess of muscular activity. The creatinine is formed essentially from the creatine. In excessive activity MONARI also found xanthocreatinine in the muscle, and the quantity was one-tenth that of the creatinine. The recent investigations of GRAHAM BROWN and CATHCART on removed nerve-muscle preparations of frogs and those of S. WEBER [3] on hearts, indicate an increase in the formation of creatine and creatinine during work. WEBER found that the working heart gave up creatine (and creatinine) to RINGER'S solution, and indeed much more when strongly active than during a lesser activity. The purine bases are, according to BURIAN,[4] increased during work, due to a greater formation (see above, page 546). It seems to have been positively shown that the active muscle contains a smaller quantity of bodies soluble in water and a larger quantity of bodies soluble in alcohol than the resting muscle. (HELMHOLTZ [5]).

Attempts have been made to solve the question relative to the behavior of the nitrogenized constituents of the muscle at rest and during activity by determining the total quantity of nitrogen eliminated under these different conditions of the body. While formerly it was held with LIEBIG that the elimination of nitrogen by the urine was increased by muscular work, the researches of several experimenters, especially those of VOIT on dogs and PETTENKOFER and VOIT on men, have led to quite different results. They have shown, as has also lately been confirmed

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[1] Siegfried, Zeitschr. f. physiol. Chem., **21**; Macleod, *ibid.*, **28**; Weyl and Zeitler, *ibid.*, **6**.

[2] Maly's Jahresber., **19**, 296.

[3] Cathcart and Graham Brown, Journ. of Physiol., **37**; Weber, Arch. f. exp. Path. u. Pharm., **58**.

[4] Zeitschr. f. physiol. Chem., **43**.

[5] Arch. f. (Anat. u.) Physiol., 1845.

by other investigators, especially I. Munk and Hirschfeld,[1] that during work no increase or only a very insignificant increase in the elimination of nitrogen takes place.

We should not omit to mention the fact that a series of experiments has been made showing a significant increase in the metabolism of proteins during or after work. There are for example the observations of Flint and of Pavy on a pedestrian, v. Wolff, v. Funke, Kreuzhage, and Kellner on a horse, and Dunlop and his collaborators on working human beings, and of Krummacher, Pflüger, Zuntz and his pupils,[2] and others. The researches on the elimination of sulphur during rest and activity also belong to this category. The elimination of nitrogen and sulphur runs parallel with the metabolism of proteins in resting and active persons, and the quantity of sulphur excreted by the urine is therefore also a measure of the protein catabolism. The earlier researches of Engelmann, Flint, and Pavy, as well as the more recent ones of Beck and Benedict,[3] and Dunlop and his collaborators, show an increased elimination of sulphur during or after work, and this indicates an increased protein metabolism because of muscular activity.

That an increased destruction of protein is not necessarily produced by work follows from the observations of Caspari, Bornstein, Kaup, Wait, A. Loewy, Atwater and Benedict,[4] that a retention of nitrogen and a deposition of protein occur during work. The discordant observations on the protein destruction during and caused by work are not directly in opposition to each other, because the extent of protein metabolism is dependent upon many conditions, such as the quantity and composition of the food, the condition of the adipose tissue of the body, the action of the work upon the respiratory mechanism, etc., all of which have an influence on the results of the experiments.

Recently Steyrer[5] has found that the muscle juice of a continuously tetanized muscle was somewhat poorer in musculin and correspondingly richer in myogen than the juice from a similar non-tetanized muscle. We cannot draw any con-

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[1] Voit, Untersuchungen über den Einfluss des Kochsalzes, des Kaffees und der Muskelbewegungen auf den Stoffwechsel (München, 1860), and Zeitschr. f. Biologie, **2**; J. Munk, Arch. f. (Anat. u.) Physiol., 1890 and 1896; Hirschfeld, Virchow's Arch., **121**.

[2] Flint, Journ. of Anat. and Physiol., **11** and **12**; Pavy, The Lancet, 1876 and 1877; v. Wolff, v. Funke, Kellner, cited from Voit, Hermann's Handb., **86**, 197; Dunlop Noel-Paton, Stockman, and Maccadam, Journ. of Physiol., **22**; Krummacher, Zeitschr. f. Biologie, **33**; Pflüger, Pflüger's Arch., **50**; Zuntz, Arch. f. (Anat. u.) Physiol., 1894.

[3] Engelmann, Arch. f. (Anat. u.) Physiol., 1871; Beck and Benedict, Pflüger's Arch., **54**, and also footnote 2.

[4] Caspari, Pflüger's Arch., **83**; Bornstein, *ibid.*; Kaup, Zeitschr. f. Biologie, **43**; Wait, U. S. Depart. Agricult. Bulletin, **89** (1901); Atwater and Benedict, *ibid.*, Bull., **69** (1899); Loewy, Arch. f. (Anat. u.) Physiol., 1901.

[5] Hofmeister's Beiträge, **4**.

clusions from this experiment, but it seems to show that the proteins are not consumed in work.

The older investigations on the amount of fat in muscles removed after activity and after rest have not led to any definite results. According to the investigations of ZUNTZ and BOGDANOW,[1] the fat belonging to the muscle-fibres, which is difficultly extracted, takes part in work. Besides these there are several researches by VOIT, PETTENKOFER and VOIT, J. FRENTZEL,[2] and others which make an increased destruction of fat during work probable or proven.

If the results of the investigations thus far made of the chemical processes going on in the active and inactive muscle were collected, we would find the following characteristics for the active muscle: The active muscle takes up more oxygen and gives off more carbon dioxide than the inactive muscle; still the elimination of carbon dioxide is increased considerably more than the absorption of oxygen. The respiratory quotient, $\frac{CO_2}{O}$, is found to be regularly raised during work; yet this rise, which will be explained in detail in a following chapter on metabolism, can hardly be conditioned on the kind of processes going on in the muscle during activity with a sufficient supply of oxygen. In work a consumption of carbohydrates, glycogen, and sugar takes place. The acid reaction of the muscle becomes greater with work. In regard to the extent of a re-formation of lactic acid opinion is divided. An increased consumption of fat has occasionally been observed. The quantity of organic phosphorus decreases, and an increase in the nitrogenous extractives of the creatinine group seems also to occur. Protein metabolism has been found increased in certain series of experiments and not in others; but an increased elimination of nitrogen as a direct consequence of muscular exertion has thus far not been positively proven.

In close connection with the above-mentioned facts there is the question as to the material basis of muscular activity so far as it has its origin in chemical processes. In the past the generally accepted opinion was that of LIEBIG, that the source of muscular action consisted of a catabolism of the protein bodies; to-day another generally accepted view prevails. FICK and WISLICENUS[3] climbed the Faulhorn and calculated the amount of mechanical force expended in the attempt. With this they compared the mechanical equivalent transformed in the same time from the proteins, calculated from the nitrogen eliminated in the

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[1] Arch. f. (Anat. u.) Physiol., 1897.

[2] Pflüger's Arch., 68.

[3] Vierteljahrsschr. d. Zürich. naturf. Gesellsch., **10**, cited from Centralbl. f. d. med. Wiss., 1866, 309.

urine, and found that the work really performed was not by any means compensated by the consumption of protein. It was therefore proven by this that proteins alone cannot be the source of muscular activity, and that this depends in great measure on the metabolism of non-nitrogenous substances. Many other observations have led to the same result, especially the experiments of VOIT, of PETTENKOFER and VOIT, and of other investigators, whose observations show that while the elimination of nitrogen remains unchanged, the elimination of carbon dioxide during work is very considerably increased. It is also generally considered as positively proven that muscular work is produced, at least in greatest part, by the catabolism of non-nitrogenous substances. Nevertheless there is no warrant for the statement that muscular activity is produced entirely at the cost of the non-nitrogenous substances, and that the protein bodies are without importance as a source of energy.

The investigations of PFLÜGER[1] are of great interest in this connection. He fed a bulldog for more than seven months with meat which alone did not contain sufficient fat and carbohydrates even for the production of heart activity, and then let him work very hard for periods of 14, 35, and 41 days. The positive result obtained by these series of experiments was that "complete muscular activity may be effected to the greatest extent in the absence of fat and carbohydrates," and the ability of proteins to serve as a source of muscular energy cannot be denied.

The nitrogenous as well as the non-nitrogenous nutriments may serve as a source of energy; but the views are divided in regard to the relative value of these. PFLÜGER claims that no muscular work takes place without a decomposition of protein, and the living cell-substance prefers always the protein and rejects the fat and sugar, contenting itself with these only when proteins are absent. Other investigators, on the contrary, believe that the muscles first draw on the supply of non-nitrogenous nutriments, and according to SEEGEN, CHAUVEAU, and LAULANIÉ[2] the sugar is indeed the only direct source of muscular force. The last-mentioned investigator holds that the fat is not directly utilized for work, but only after a previous conversion into sugar. ZUNTZ and his collaborators have made strong objections to the correctness of such a view. If, according to ZUNTZ, the fat must be first transformed into sugar before it can serve as the source of muscular work, a definite expenditure of force must require about 30 per cent more energy with fatty food than it does with carbohydrates; but this is not the case. The investigations

[1] Pflüger's Arch., **50**.

[2] See Seegen, footnote 4, page 563. The works of Chauveau and his collaborators are found in Compt. rend., **121**, **122**, and **123**; Laulanié, Arch. de Physiol. (5), 8.

of ZUNTZ, (together with) LOEB, HEINEMANN, FRENTZEL and REACH show that all foodstuffs have nearly the same power of serving as the material for the work of the muscles. The extensive metabolism investigations of ATWATER and BENEDICT[1] have also led to similar results as to the fats being a source of muscular energy. The law of the substitution of the foodstuffs, according to their combustion equivalents, is also true for muscular work, and fat correspondingly acts with its full amount of energy without previously being transformed into sugar. The question which of the foodstuffs the muscle prefers is dependent upon the relative quantities of the same at the disposal of the muscle. A direct substitution of the body material by the bodies supplied as food does not take place in the muscular activity in the ordinary nutritive condition. According to JOHANSSON and KORAEN[2] the CO_2 excretion produced by certain work is not influenced by the supply of foodstuffs (protein or sugar).

SIEGFRIED considers, as above stated, the phosphocarnic acid as a source of energy. According to his and KRÜGER'S[3] researches, phosphocarnic acid, which yields on cleavage, among other bodies, carbon dioxide, occurs in part preformed in the muscle, and in part as a hypothetical aldehyde compound of the same—a compound which forms phosphocarnic acid on oxidation. SIEGFRIED therefore makes the suggestion that in the resting muscle, which requires more oxygen than exists in the carbon dioxide eliminated, this reducing aldehyde substance is gradually oxidized to phosphocarnic acid, which is used in the activity of the muscle with the splitting off of carbon dioxide.

Quantitative Composition of the Muscle. A large number of analyses have been made of the flesh of various animals for purely practical purposes, in order to determine the nutritive value of different varieties of meat; but there are no exact scientific analyses with sufficient regard to the quantity of different protein bodies and the remaining muscle constituents, that is, these analyses are incomplete or of little value.

To give the reader some idea of the variable composition of muscle-substance the following summary is presented, chiefly obtained from K. B. HOFMANN'S[4] book, although it does not correspond to the present demands. The figures are parts per 1000.

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[1] Loeb, Arch. f. (Anat. u.) Physiol., 1894; Heinemann, Pflüger's Arch., **83**; Frentzel and Reach, *ibid.*; Atwater and Benedict, U. S. Dept. of Agric., Bull. **136**, and Ergebnisse der Physiologie, **3**.

[2] Skand. Arch. f. Physiol., **13**.

[3] Zeitschr. f. physiol. Chem., **22**.

[4] Lehrbuch d. Zoochemie (Wien, 1876), 104.

	Muscles of Mammals.	Muscles of Birds.	Muscles of Cold-blooded Animals.
Solids	217–255	225–282	200
Water	745–783	717–773	800
Organic bodies	208–245	217–263	180–190
Inorganic bodies	9–10	10–19	10–20
Myosin	35–106	29.8–111	29.7–87
Stroma substance (Danilewsky)	78–161	88.0–184	70.0–121
Creatine	2–4	4.9	2.3
Xanthine bodies	1.3–1.7	0.7–1.3	—
Inosinic acid (barium salt)	0.1	0.1–0.3	—
Protic acid	—	—	7.0
Taurine	0.7 (horse)	—	1.1
Inosite	0.03	—	—
Glycogen	4–37	—	3–5
Lactic acid	0.4–0.7	—	—
Phosphoric acid	3.4–4.8		
Potash	3.0–4.0		
Soda	0.3		
Lime	0.2		
Magnesia	0.4		
Sodium chloride	0.04–0.1		
Iron oxide	0.04–0.1		

In this table, which has little value because of the variation in the composition of the muscles, no results are given as to the estimates of *fat*. Owing to the variable quantity of fat in meat and the incompleteness of the older methods of estimation, it is hardly possible to quote a positive average for this substance. After most careful efforts to remove the fat from the muscles without chemical means, it has been found that a variable quantity of intermuscular fat, which does not really belong to the muscular tissue, always remains. The smallest quantity of fat in the muscles from lean oxen is 6.1 p. m. according to Grouven, and 7.6 p. m. according to Petersen. This last observer also regularly found a smaller quantity of fat, 7.6–8.6 p. m., in the fore quarters of oxen, and a greater amount, 30.1–34.6 p. m., in the hind quarters of the animal, but this could not be substantiated by Steil.[1] A small quantity of fat has also been found in the muscle of wild animals. B. König and Farwick found 10.7 p. m. fat in the muscles of the extremities of the hare, and 14.3 p. m. in the muscles of the partridge. The muscles of pigs and fattened animals are, when all the adherent fat is removed, very rich in fat, amounting to 40–90 p. m. The muscles of certain fishes also contain a large quantity of fat. According to Almén, in the flesh of the salmon, the mackerel, and the eel there are contained respectively 100, 164, and 329 p. m. fat.[2]

[1] See Steil, Pflüger's Arch., **61**.

[2] In regard to the literature and complete reports on the composition of flesh of various animals, see König, Chemie der menschlichen Nahrungs- und Genussmittel, 5. Aufl.

The quantity of *water* in the muscle is liable to considerable variation. The quantity of fat has a special influence on the quantity of water, and one finds, as a rule, that the flesh which is deficient in water is correspondingly rich in fat. The quantity of water does not depend upon the amount of fat alone, but upon many other circumstances, among which must be mentioned the age of the animal. In young animals, the organs in general, and therefore also the muscles, are poorer in solids and richer in water. In man the quantity of water decreases until mature age, but increases again toward old age. Work and rest also influence the quantity of water, for the active muscle contains more water than the inactive. The uninterruptedly active heart should therefore be the muscle richest in water. That the quantity of water may vary independently of the amount of fat is strikingly shown by comparing the muscles of different species of animals. In cold-blooded animals the muscles generally have a greater quantity of water, in birds a lower. The comparison of the flesh of cattle and fish shows very strikingly the different amounts of water (independent of the quantity of fat) in the flesh of different animals. According to the analysis of ALMÉN[1] the muscles of lean oxen contain 15 p. m. fat and 767 p. m. water; the flesh of the pike contains only 1.5 p. m. fat and 839 p. m. water.

For certain purposes, as, for example, in experiments on metabolism, it is important to know the elementary composition of flesh. In regard to the quantity of nitrogen we generally accept VOIT's figure, namely, 3.4 per cent, as an average for fresh lean meat. According to NOWAK and HUPPERT[2] this quantity may vary about 0.6 per cent, and in more exact investigations it is therefore necessary to specially determine the nitrogen. Complete elementary analyses of flesh have been made with great care by ARGUTINSKY. The average for ox-flesh dried *in vacuo* and free from fat and with the glycogen deducted was as follows: C 49.6; H 6.9; N 15.3; O+S 23.0; and ash 5.2 per cent. KÖHLER found as an average for water and fat-free beef C 49.86; H 6.78; N 15.68; O+S 22.3 per cent, which are very similar results. This investigator also made similar analyses of the flesh of various animals and determined the calorific value of the ash- and fat-free dried meat substance. This value was, per gram of substance, 5599–5677 cal. The relation of the carbon to nitrogen, which ARGUTINSKY calls the "*flesh quotient*," is on an average 3.24:1. From KÖHLER's analyses the average for beef is 3.15:1 and for horse-flesh 3.38:1. MAX MÜLLER has shown with experiments on dogs, that the flesh of the same individual shows some

[1] Nova Act. Reg. Soc. Scient. Upsal., Vol. extr. ord., 1877; also Maly's Jahresber., 7.

[2] Voit, Zeitschr. f. Biologie, **1**; Huppert, *ibid*, **7**; Nowak, Wien. Sitzungsber., **64**, Abt. 2.

variation in this quotient after different foods. According to SALKOWSKI, of the total nitrogen of beef 77.4 per cent was insoluble proteins, 10.08 per cent soluble proteins, and 12.52 per cent other soluble bodies. FRENTZEL and SCHREUER[1] find that about 7.74 per cent of the total nitrogen belongs to the nitrogeous extractives.

There exist complete investigations by KATZ[2] as to the quantity of mineral constituents of the muscles from man and animals. The variation in the different elements is considerable. Pork is much richer in sodium as compared with potassium than other kinds of meat. The quantity of magnesium is greater, and often considerably greater, than calcium in all kinds of flesh investigated, with the exception of the haddock, the eel, and the pike. Beef is very poor in calcium. Potassium and phosphoric acid are the most abundant mineral constituents of all flesh.

Non-striated Muscles.

The smooth muscles have a neutral or alkaline reaction (DUBOIS-REYMOND) when at rest. During activity they are acid, which is inferred from the observations of BERNSTEIN, who found that the almost continually contracting sphincter muscle of the Anodonta is acid during life. The smooth muscles may also, according to HEIDENHAIN and KÜHNE,[3] pass into rigor mortis and thereby become acid. A spontaneous but slowly coagulating plasma has also been observed in several cases.

In regard to the proteins of the smooth muscles we have the earlier accounts of HEIDENHAIN and HELWIG;[4] but they were first carefully studied according to newer methods by MUNK and VELICHI.[5] These experimenters prepared a neutral plasma from the gizzard of geese, according to v. FÜRTH'S method. This plasma coagulated spontaneously at the temperature of the room, although slowly. It contained a *globulin*, precipitated by dialysis, which coagulated at 55–60° C. and also showed certain similarities with KÜHNE'S myosin. A spontaneously coagulating *albumin*, which differed from myogen (v. FÜRTH) by coagulating at 45–50° C., and which passes by spontaneous coagulation into the coagulated modification without a soluble intermediate

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[1] Argutinsky, Pflüger's Arch., **55**; Köhler, Zeitschr. f. physiol. Chem., **31**; Salkowski, Centralbl. f. d. med. Wissensch., 1894; Frentzel and Schreuer, Arch. f. (Anat. u.) Physiol., 1902; Müller, Pflüger's Arch., **116**.

[2] Pflüger's Arch., **63**. See also Schmey, Zeitschr. f. physiol. Chem., **39**.

[3] Du Bois-Reymond in Nasse, Hermann's Handb., **1**, 339; Bernstein, *ibid.*, Heidenhain, *ibid.*, 340, with Hellwig, *ibid.*, 339; Kühne, Lehrbuch, 331.

[4] Heidenhain in Nasse, Hermann's Handb., **1**, 340, with Hellwig, *ibid.*, 339; Kühne, Lehrbuch, 331.

[5] Munk and Velichi, Centralbl. f. Physiol., **12**.

product, exists in still greater quantities in this plasma. Alkali albuminates do not occur, but a *nucleoprotein* is found, which exists in about five times the quantity as compared with striated muscles. Nucleon is, according to PANELLA,[1] a normal constituent of smooth muscles and occurs in larger amounts than in striated muscles.

Recent investigations of BOTTAZZI and CAPPELLI, VINCENT and LEWIS, VINCENT and v. FÜRTH,[2] some on the muscles of warm-blooded and some on those of lower animals, have led to dissimilar results, but they substantiate, as a whole, the observations of MUNK and VELICHI. Besides the nucleoproteins the smooth muscles contain two bodies corresponding in coagulation temperature to musculin and myosinogen (myogen, v. FÜRTH), but they are not identical therewith. *Hæmoglobin* occurs in the smooth muscles of certain animals, but is absent in others. In the smooth muscles (in certain varieties of animals) *creatine, creatinine, hypoxanthine, taurine, inosite, glycogen,* and *lactic acid* have been found. The mineral constituents show the remarkable fact that the sodium compounds exceed the potassium compounds. According to SAIKI[3] magnesium does not occur to a greater extent than calcium in the smooth muscles of the stomach or the bladder of pigs. The same investigator found 801–811 p. m. water and 199–189 p. m. solids in these muscles.

HENZE found abundance of taurine in the muscles of Octopods, 5 p. m., but no creatine, which, according to FRÉMY and VALENCIENNES,[4] occurs in the muscles of Cephalopods. He also found no glycogen and no paralactic acid, but, on the contrary, small amounts of fermentation lactic acid. The muscles of Octopods are richer in mineral bodies than the muscles of vetebrates, and are nearly twice as rich in sulphur as these.

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[1] Maly's Jahresber., **34**.

[2] Bottazzi, Centralbl. f. Physiol., **15**; Vincent and Lewis, Journ. of Physiol., **26**; Vincent, Zeitschr. f. physiol. Chem., **34**; v. Fürth, *ibid.*, **31**.

[3] Journ. of Biol. Chem., **4**.

[4] Henze, *ibid.*, **43**; Frémy and Valenciennes, cited from Kühne's Lehrbuch, p. 333.

CHAPTER XII.

BRAIN AND NERVES.

On account of the difficulty in making a mechanical separation and isolation of the different tissue-elements of the central nervous organ and the nerves, we must resort to a few microchemical reactions, chiefly to qualitative and quantitative investigations of the different parts of the brain, in order to study the varied chemical composition of the cells and the nerve-axes. This study is accompanied with the greatest difficulty, and although our knowledge of the chemical composition of the brain and nerves has been somewhat extended by the investigations of modern times, still it must be admitted that this subject is as yet one of the most obscure and complicated in physiological chemistry.

Proteins of different kinds have been shown to be chemical constituents of the brain and nerves, and these are representatives of the same chief groups as occur in the protoplasm. In the brain there occur some proteins which are insoluble in water and neutral salt solutions, and which resemble the stroma substances of the muscles and cells, while other proteins are soluble in water and neutral salt solutions. Among the latter we find chiefly *nucleoproteins* and *globulins*. The nucleoprotein found by Halliburton and also by Levene[1] in the gray substance contains 0.5 per cent phosphorus and coagulates at 55–60°. Levene obtained adenine and guanine but no hypoxanthine as cleavage products. According to Halliburton there are two globulins, namely, the neuroglobulin α, which coagulates at 47°, or at, in the case of birds 50–53°, and the neuroglobulin β, whose coagulation temperature is 70–75°, but which varies somewhat in different animals. In the frog still another protein body occurs, which coagulates at a still lower temperature, about 40°. It must be remarked that the coagulation temperature of α-globulin corresponds with the temperature of the first heat contraction of the nerves of different classes of animals (Halliburton).

There does not seem to be any doubt that the proteins chiefly belong to the gray substance of the brain and to the axis-cylinders. The same

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Halliburton, On the Chemical Physiology of the Animal's Cell, King's College, London, Physiological Laboratory, Collected Papers No. 1, 1893, and Ergebnisse der Physiologie, 4; Levene, Arch. of Neurology and Psychopathology, 2 (1899).

remark also applies to the *nuclein*, which v. JACKSCH [1] found in large quantities in the gray substance. *Neurokeratin*, which was first detected by KÜHNE, and which partly forms the *neurolgia*, and as a double sheath envelops the outside of the nerve-medulla under SCHWANN'S sheath and the inner axis-cylinders, occurs in the nerves, but chiefly, or according to KOCH entirely, in the white substance (KÜHNE and CHITTENDEN, BAUMSTARK [2]).

The so-called *protagon* has been considered as one of the chief constituents, perhaps the only constituent (BAUMSTARK), of the white substance. This protagon, according to most investigators, is only a mixture of phosphatides with the non-phosphorized *cerebron* or with a mixture of cerebrosides (see below). There does not seem to be any doubt that these last, especially the cerebron as well as *lecithin* and *cephalin*, occur preformed in the brain and nerves. From the investigations made thus far it is difficult to state with positiveness whether the last two mentioned bodies (which have been discussed in Chapter V) belong to the gray or white substance. According to KOCH they occur much more abundantly in the white substance. FALK [3] prepared a phosphatide from human brain, which behaves essentially like cephalin and also yields cephalinic acid, which is difficult of characterization. The strikingly high nitrogen content is remarkable, namely in one preparation 2.905 per cent and 2.76 per cent in another. The phosphorus content was 3.28 and 3.16 per cent. The relation P:N is correspondingly about 1:2. The isolated substance is a diamido-phosphatide while the cephalin thus far investigated is a monamido-phosphatide. Under these circumstances, irrespective of the purity and the chemical identity of the isolated substance, we are not warranted in calling this substance cephalin, as was done by FALK. On the other hand it is also questionable whether we are warranted in including cephalin among the lecithins as done in the text on page 234. The observations of KOCH [4] show that at least the human and ox brain contain *jecorin*, and that the lipoid sulphur occurs, much more abundantly, in the white than in the gray substance. The same is true for the numerous *brain phosphatides* described by THUDICHUM [5] under different names, and whose chemical individuality has not been sufficiently established; *cholesterin* chiefly occurs in the white substance. *Fatty acids* and *neutral fats* may

[1] Pflüger's Arch., **13**.

[2] Koch, Amer. Journ. of Physiol., **11**; Kühne and Chittenden, Zeitschr. f. Biologie, **26**; Baumstark, Zeitschr. f. physiol. Chem., **9**.

[3] Bioch. Zeitschr., **16**.

[4] Zeitschr. f. physiol. Chem., **53**.

[5] Thudichum, Die chemische Konstitution des Gehirns des Menschen und der Tiere, Tübingen, 1901.

be prepared from the brain and nerves; but as these may be readily derived from a decomposition of phosphatides, which exist in the fatty tissue between the nerve-axes, it is difficult to decide what part the fatty acids and neutral fats play as constituents of the real nerve-substance.

By allowing water to act on the contents of the medulla, round or oblong double-contoured drops or fibres, not unlike double-contoured nerves, are formed. These remarkable formations, which can also be seen in the medulla of the dead nerve, have been called "*myeline forms*," and they were formerly considered as produced from a special body, "myeline." Myeline forms may, however, be obtained from other bodies, such as impure protagon, lecithin, and impure cholesterin, and they depend upon a decomposition of the constituents of the medulla.

The *extractive bodies* seem to be almost the same as in the muscles. One finds *creatine*, which may, however, be absent (BAUMSTARK), *purine bases, inosite, choline, paralactic acid* (MORIYA), *phosphocarnic acid, uric acid*, and the diamine *neuridine*, $C_5H_{14}N_2$, discovered by BRIEGER[1] and which is most interesting because of its appearance in the putrefaction of animal tissues or in cultures of the typhoid bacillus. Under pathological conditions *leucine* and *urea* have been found in the brain. Urea is also a physiological constituent of the brain of cartilaginous fishes.

Of the above-mentioned constituents of the nerve-substance protagon and the cerebrins or cerebrosides must be specially described.

Protagon. Under this name LIEBREICH described a crystalline, nitrogenous and phosphorized substance, which has been found in the brain of man, mammalia and also birds (ARGIRIS) but not in the brain of fishes (ARGIRIS). Its elementary composition, according to GAMGEE and BLANKENKORN is C 66.39, H 10.69, N 2.39 and P 1.07 per cent. The results for carbon and phosphorus, namely 66.25 and 0.97 per cent, found by KOSSEL and FREYTAG, correspond well with these figures while the result for the nitrogen, 3.25 per cent, is too high. They also found, as shown previously by RUPPEL, that protagon contains sulphur, namely 0.51 per cent. CRAMER also found that the protagon contained sulphur, but obtained about the same figures as GAMGEE and BLANKENHORN. Recently WILSON and CRAMER[2] have reported newer analyses and they find for protagon, recrystallized 4–5 times, nearly the same figures as GAMGEE and BLANKENHORN, namely C 66.53, H 10.97, P 0.95 and S 0.73 per cent. They consider protagon as a unit substance.

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[1] Brieger, Ueber Ptomaine, Berlin, 1885 and 1886.

[2] Lieberich, Annal. d. Chem. u. Pharm., **134**; Argiris, Zeitschr. f. physiol. Chem., **57**; Gamgee and Blankenhorn, *ibid.*, **3**; Kossel and Freytag, *ibid.*, **17**; Ruppel, Zeitschr. f. Biol., **31**; Cramer, Journ. of Physiol., **31**, with R. A. Wilson, Journ. of exp. Physiol., **1**, with Lockhead, Bioch. Journ., **2**.

GIES, POSNER and ROSENHEIM and TEBB [1] dispute the unit nature of protagon. They have found, on fractional precipitation or on recrystallization, that protagons can be obtained from the various solvents, having variable composition, especially different P and N contents. They are, therefore, as are LESEM, THUDICHUM, WÖRNER and THIERFELDER,[2] and others, of the opinion that protagon does not exist as a chemical individual, but as a mixture of cerebrosides and phosphatides. It is not easy to come to any decision on this disputed question. The above-mentioned investigations, and especially the recent ones of ROSENHEIM and TEBB, imply the absence of unity of protagon but do not exclude the possibility that protagon is a loose chemical combination between cerebroside and phosphatide, which like other readily dissociable combinations, exist only under certain conditions or in certain solvents. It is difficult to understand how a mixture of amorphous or only difficultly crystallizable bodies can be so easily crystallized and yield a product, which with proper care, can be recrystallized repeatedly without changing its composition. According to ROSENHEIM and TEBB if the proper quantity is used in solution a crystalline product can be obtained from the decomposition products of protagon, which has the same specific rotation as protagon and can be repeatedly recrystallized without changing its composition or its optical activity.[3] If this is the case it would be better to investigate the cleavage products than to analyze the various fractions in order to get information on the disputed question.

As we are not decided whether protagon is only a mixture or is a body contaminated with other substances, it is difficult to decide as to how far the so-called decomposition products exist as preformed constituents of the mixture or whether they are true decomposition products. On boiling with baryta-water protagon yields *cerebrosides* (see below) and the decomposition products of lecithin, namely, fatty acids, glycerophosphoric acid, and choline. KOSSEL and FREYTAG indeed found three cerebrosides, namely, CEREBRIN, KERASIN (homocerebrin), and ENCEPHALIN. According to KOCH [4] the protagon molecule contains cerebroside, lecithin and sulphuric acid (in ester-like combination with the cerebroside) besides excess of cerebroside. Of interest is the finding of KITAGAWA and THIERFELDER [5] that protagon dissolved in methyl alcohol containing chloroform, deposits crusts of cerebron (not pure) after a time at

[1] Gies and Lesem, Amer. Journ. of Physiol., 8; Posner and Gies, Journ. of biol. Chem., 1; Gies, *ibid.*, 3; Rosenheim and Tebb, Journ. of Physiol., 36 and 37.

[2] Lesem, l. c.; Thudichum, l. c.; Wörner and Thierfelder, Zeitschr. f. physiol. Chem., 30.

[3] Journ. of Physiol., 37; Proc. physiol. Soc., January, 1908, p. 3.

[4] Zeitschr. f. physiol. Chem., 53.

[5] Kitagawa and Thierfelder, *ibid.*, 49; Rosenheim and Tebb, Journ. of Physiol., 37, 341 and 348.

ordinary temperature, and that as shown by ROSENHEIM and TEBB, on dissolving in pyridine at 30° C. and heating or cooling the solution deposits a precipitate of a substance rich in phosphorus. Although we generally consider the phosphorized component of protagon as lecithin, still, according to ROSENHEIM and TEBB it is probably a diamido-phosphatide, called *sphingomyelin* by THUDICHUM. On boiling protagon with dilute mineral acids it yields a reducing sugar (galactose) due to the decomposition of the cerebrosides.

Protagon appears, when dry, as a loose white powder. It dissolves in alcohol of 85 vols. per cent at 45° C., but separates on cooling as a snow-white, flaky precipitate, consisting of globules or groups of fine crystalline needles. It decomposes on heating even below 100° C. It is difficultly soluble in cold alcohol or ether, but dissolves, at least when freshly precipitated, in ether on warming. It dissolves in methyl alcohol containing chloroform and, as above stated, separates cerebroside. Protagon is soluble in pyridine at 30° C., yielding a clear solution, and this solution has a specific rotation $(\alpha)_D = +6.8°$ (WILSON and CRAMER). On warming or cooling according to ROSENHEIM and TEBB, the rotation changes with the separation of sphingomyelin so that it first diminishes in rotation, then is zero, and then becomes strongly levorotatory until it reaches $-242°$, and finally, when nearly all the sphingomyelin has separated out it becomes constant at about $-13.3°$. The strong levorotation depends upon the accumulations of doubly refracting spheroid crystals of sphingomyelin. With little water protagon swells up and is partly decomposed. With more water it forms a jelly or pasty-like mass which, with the addition of considerable water, forms an opalescent liquid. On fusion with saltpeter and soda it yields alkali phosphate.

Protagon can be prepared in the following way: The finely ground brain-mass, as free as possible from blood and membrane, is dehydrated, which is best done by cold acetone or by grinding with burnt plaster-of-paris or anhydrous sodium sulphate, and then extracted with ether. The mass is then extracted at 45° C. with 85 vol. per cent alcohol until the filtrate when cooled to 0° C. gives no more precipitate. All the precipitates obtained on cooling to 0° C. are extracted with ether and recrystallized from alcohol. Further details can be found in the cited works of CRAMER, WILSON, GIES, ROSENHEIM and TEBB.

Cerebrosides or Cerebrins.

On decomposing protagon (or the protagons) by the gentle action of alkalies we obtain, as cleavage products, as above stated, one or more bodies which THUDICHUM has embraced under the name *cerebrosides*. The cerebrosides are nitrogenous substances free from phosphorus, which yield a reducing variety of sugar (galactose) on boiling with dilute mineral

acids. The cerebrosides isolated from the brain are cerebrin, kerasin, encephalin, and cerebron, but it must be remarked that there is no doubt that sometimes the same body of varying purity has received different names. The bodies isolated by KOSSEL and FREYTAG from pus, and called pyosin and pyogenin, also belong to the cerebrosides (see Chapter VII).

Cerebrin. Under this name W. MÜLLER[1] first described a nitrogenous substance, free from phosphorus, which he obtained by extracting, with boiling alcohol, a brain-mass which had been previously boiled with baryta-water. Following a method essentially the same, but differing slightly, GEOGHEGAN[2] prepared, from the brain, a cerebrin with the same properties as MÜLLER'S, but containing less nitrogen. According to PARCUS[3] the cerebrin isolated by GEOGHEGAN, as well as by MÜLLER, consists of a mixture of three bodies, "cerebrin," "homocerebrin," and "encephalin." KOSSEL and FREYTAG isolated two cerebrosides from protagon which were identical with the cerebrin and homocerebrin of PARCUS. According to these investigators, the two bodies phrenosin and kerasin, as described by THUDICHUM, seem to be identical with cerebrin and homocerebrin.

Cerebrin, according to PARCUS, has the following composition: C 69.08, H 11.47, N 2.13, O 17.32 per cent, which corresponds with the analyses made by KOSSEL and FREYTAG. No formula has been given to this body. In the dry state it forms a pure white, odorless, and tasteless powder. On heating it melts, decomposes gradually, smells like burnt fat, and burns with a luminous flame. It is insoluble in water, dilute alkalies, or baryta-water; also in cold alcohol and in cold or hot ether. On the contrary, it is soluble in boiling alcohol and separates as a flaky precipitate on cooling, and this is found to consist of a mass of globules or grains on microscopical examination. Cerebrin forms a compound with baryta, which is insoluble in water and is decomposed by the action of carbon dioxide. Cerebrin dissolves in concentrated sulphuric acid, and on warming the solution it becomes blood-red. The variety of sugar split off on boiling with mineral acids—the so-called brain-sugar—is, in THIERFELDER'S[4] opinion, galactose.

Kerasin (THUDICHUM), or *homocerebrin* (PARCUS), has the following composition: C 70.06, H 11.60, N 2.23, and O 16.11 per cent. **Encephalin** has the composition C 68.40, H 11.60, N 3.09, and O 16.91 per cent. Both bodies remain in the mother-liquor after the impure cerebrin has precipitated from the warm alcohol. These bodies have the tendency of separating as gelatinous masses. Kerasin is similar to cerebrin, but

[1] Annal. d. Chem. u. Pharm., **105**.
[2] Zeitschr. f. physiol. Chem., **3**.
[3] Parcus, Ueber einige neue Gehrinstoffe, Inaug.-Diss. Leipzig, 1881.
[4] Zeitschr. f. physiol. Chem., **14**.

dissolves more easily in warm alcohol and also in warm ether. It may be obtained as extremely fine needles. Encephalin is, PARCUS thinks, a transformation product of cerebrin. In the perfectly pure state it crystallizes in small lamellæ. It swells in warm water into a pasty mass. Like cerebrin and kerasin, it yields a reducing substance (probably galactose) on boiling with dilute acid.

As the purity and the chemical individuality of the above-mentioned bodies is questionable, it is perhaps sufficient in regard to their preparation to simply call attention to the cited works of MÜLLER, GEOGHEGAN, KOSSEL and FREYTAG. All these methods split with barium hydroxide and purify the cerebroside by solution in hot alcohol and a precipitation by cooling.

Whether the above-described cerebrosides are chemical individuals or mixtures, i.e., impure substances, is still undecided. The purest cerebroside thus far investigated is undoubtedly THIERFELDER'S cerebron, and there is hardly any doubt that the above-mentioned cerebrosides consist essentially of this body.

Cerebron. This cerebrin, isolated by THIERFELDER and WÖRNER and then especially studied by THIERFELDER, was first isolated by GAMGEE and called *pseudocerebrin* by him. THUDICHUM'S *phrenosin* is, according to GIES,[1] identical with cerebron. Cerebron can be prepared directly from the brain without saponification with baryta, by treatment with alcohol containing benzene or chloroform at a temperature of 50°, and hence it is considered as existing preformed in the brain. According to THIERFELDER cerebron has the formula $C_{43}H_{93}NO_9$; it melts at 212°, dissolves in warm alcohol, and separates out on cooling. From proper solvents (acetone or methyl alcohol containing chloroform) it may be separated as small needles or plates. If cerebron is suspended in 85-per cent alcohol at a temperature of 50° C. it balls together in amorphous masses, and from these needle- and leaf-shaped crystals gradually form. It is dextrorotatory, and in about a 5-per cent solution in methyl alcohol (containing 75 per cent chloroform) is $(\alpha)_D = +7.6°$ (KITAGAWA and THIERFELDER). According to THIERFELDER it yields as cleavage products, *galactose*, *cerebronic acid* (THUDICHUM "neurostearic acid") and a mixture of two bases, which have not been closely studied, of which one is crystalline, gives a beautiful crystalline compound with HCl and has the probable composition $C_{19}H_{39}NO_2$. The relation of these bases to THUDICHUM'S base sphingosin has not been explained. Cerebronic acid is crystalline, melts at 99–100° C. and gives a crystalline methyl ester melting at 65° C.

Cerebron can best be prepared, according to THIERFELDER and KITAGAWA, by decomposing the protagon in methyl alcohol containing

[1] Thierfelder and Wörner, Zeitschr. f. physiol. Chem., **30**; Thierfelder, *ibid.*, **43**, **44**, **46**, with Kitagawa, *ibid.*, **49**; Gamgee, Text-book of Physiol. Chem., London, 1880; Thudichum, l. c.; Gies, Journ. of Biol. Chem., **1** and **2**.

chloroform (see page 577), and purifying the separated cerebron from contaminating phosphatides by precipitating these with an ammoniacal solution of zinc hydroxide in methyl alcohol and recrystallizing the cerebron from methyl alcohol containing chloroform.

The two monamido-monophosphatides, lecithin and cephalin, have already been discussed in Chapter V.

BETHE [1] prepared the following decomposition products from the brain of the horse after treatment with $CuCl_2$ and alkali: *Aminocerebrinic-acid glucoside*, $C_{44}H_{81}O_8N$, which on boiling with hydrochloric acid yields cerebrinic acid, aminocerebrinic-acid chloride, and a hexose (galactose ?); *phrenin*, perhaps identical with THUDICHUM'S krinosin; *cerebrinic-phosphoric acid*, and a *stearic acid*, differing somewhat from the ordinary one.

Neuridine, $C_5H_{14}N_2$, is a non-poisonous diamine discovered by BRIEGER, and obtained by him in the putrefaction of meat and gelatin, and from cultures of the typhoid bacillus. It also occurs under physiological conditions in the brain, and as traces in the yolk of the egg.

Neuridine dissolves in water and yields on boiling with alkalies a mixture of dimethylamine and trimethylamine. It dissolves with difficulty in amyl alcohol. It is insoluble in ether or absolute alcohol. In the free state, neuridine has a peculiar odor, suggesting semen. With hydrochloric acid it gives a compound crystallizing in long needles. With platinic chloride or gold chloride it gives crystallizable double compounds which are valuable in its preparation and detection.

The so-called CORPUSCULA AMYLACEA, which occur on the upper surface of the brain and in the pituitary gland, are colored more or less pure violet by iodine and more blue by sulphuric acid and iodine. They perhaps consist of the same substance as certain prostatic calculi, but they have not been closely investigated.

Quantitative Composition of the Brain. The quantity of water is greater in the gray than in the white substance, and greater in new-born or young individuals than in adults. The brain of the fœtus contains 879–926 p. m. water. The observations of WEISBACH [2] show that the quantity of water in the several parts of the brain (and in the medulla) varies at different ages. The following figures are in 1000 parts—*A* for men and *B* for women:

	20–30 years.		30–50 years.		50–70 years.		70–94 Years.	
	A.	*B.*	*A.*	*B.*	*A.*	*B.*	*A.*	*B.*
White brain-substance..	695.6	682.9	683.1	703.1	701.9	689.6	726.1	722.0
Gray " .	833.6	826.2	836.1	830.6	838.0	838.4	847.8	839.5
Gyri.....	784.7	792.0	795.9	772.9	796.1	796.9	802.3	801.7
Cerebellum.....	788.3	794.9	778.7	789.0	787.9	784.5	803.4	797.9
Pons Varolii.....	734.6	740.3	725.5	722.0	720.1	714.0	727.4	724.4
Medulla oblongata.....	744.3	740.7	732.5	729.8	722.4	730.6	736.2	733.7

Quantitative analyses of human brains at different ages, namely 6 weeks, 2 and 19 years, have been made by KOCH and MANN.[2] These analyses show that with increasing age the water, proteins, extractives and salts diminish relatively, while the phosphatides, cerebrosides and especially cholesterin strikingly increase. The sulphur of the lipoids is

[1] Arch. f. exp. Path. u. Pharm., 48.

[2] Cited from K. B. Hoffmann's Lehrbuch d. Zooch., Wien, 1877, p. 121.

[3] Journ. of Physiol., 36, Proc. physiol. Soc., 1907.

increased until the second year, but from this age to the nineteenth year the quantity was the same.

The older analyses of PETROWSKY and of BAUMANN [1] are not sufficient for present conditions, but they substantiate in substance what has been stated (page 575) in regard to the unequal division of the organic constituents between the gray and white substance.

BAUMSTARK claims to have found that a part of the cholesterin in the brain occurs in a combined state, perhaps as ester; this view has been found to be incorrect by the recent investigations of BÜNZ. He obtained from the brain neither esters of cholesterin with higher fatty acids nor other compounds of cholesterin which split on saponification. TEBB [2] has also found only free cholesterin.

The analysis of the brain of an epileptic made by KOCH [3] is of very great interest. As the protagon is considered by KOCH as a mixture, no results for the quantity of protagon are given. As no accurate methods for the estimation of the little known bodies cephalin, myelin, phrenosin and kerasin are available, the figures given for these are of little value. The following results are calculated to 1000 parts:

	Corpus Callosum		Cortex (prefrontal).	
Water	679.7		841.3	
Protein	32.0		50.0	
Nucleoproteins	37.0		30.0	
Nuerokeratin	27.0	(CHITTENDEN)	4.0	(CHITTENDEN)
Extractives (water-soluble)	15.1		15.8	
Lecithins	51.9		31.4	
Cephalin and myelin	34.9		7.4	
Phrenosin and kerasin	45.7		15.5	
Cholesterin	48.6		7.0	
Sulphurized substance	14.0		14.5	
Mineral bodies	8.2		8.7	

As the cerebrosides chiefly occur in the myelin sheath, KOCH, starting from the amount in the investigated part of the brain, attempts to calculate the amount of the analyzed cortical substance in the white nerves, and on the basis of these calculations, he finds the following values for the pure gray substance, free from nerve-fibres, and compares them with the corpus callosum. The results are in 1000 parts of the dry substance:

	Corpus Callosum.	Gray Substance (free from white substance).
Protein	100.0	217.0
Nucleoproteins	115.6	96.6
Neurokeratin	84.0	—
Extractives	47.5	59.2
Lecithins	162.2	76.7
Cephalin and myelin	109.1	—
Phrenosin and kerasin	142.9	—
Cholesterin	152.0	—
Sulphurized substance	43.7	54.3

[1] Petrowsky, Pflüger's Arch., 7; Baumann, Zeitschr. f. physiol. Chem., 9.

[2] Bünz, Zeitschr. f. physiol. Chem., 46; Tebb, Journ. of Physiol., 34.

[3] Amer. Journ. of Physiol., 11.

The above assumption has not sufficient foundation and is less probable, as FALK [1] has found cerebrosides in the medullary nerve fibres as well as in nerves without medullas. These latter yielded much less substance on extraction than the medullary, namely 11.51 per cent extract as compared to 46.59 per cent. The extract of the first was poorer in cerebrosides, but richer in cholesterin, cephalin and lecithin, as shown by the following figures.

	Non-medullary fibres in p. m. of the total extract	Medullary fibres in p. m. of the total extract
Cholesterin	470	250
Cephalin	237	124
Cerebrosides	60	182
Lecithins	98	29

According to NOLL the white substance of the spinal marrow is somewhat richer in protagon than the brain, and in nerve degeneration the quantity of protagon diminishes. The method used by him would not allow of an exact determination of the disputed substance protagon. MOTT and HALLIBURTON [2] have also shown that in degenerative diseases of the nervous system the quantity of substances containing phosphorus diminishes, and that in these cases, especially in general paralysis, choline passes into the cerebrospinal fluid and the blood. In degenerated nerves, the quantity of water increases and the phosphorus decreases. On comparative investigations of the central nervous system of normal persons and those afflicted with dementia præcox (5 cases), KOCH [3] found that the variation from the normal composition was not great enough nor so constant that positive conclusions could be drawn therefrom.

The quantity of neurokeratin in the nerves and the different parts of the brain has been carefully determined by KÜHNE and CHITTENDEN.[4] They found 3.16 p. m. in the plexus brachialis, 3.12 p. m. in the cortex of the cerebellum, 22.434 p. m. in the white substance of the cerebrum, 25.72–29.02 p. m. in the white substance of the corpus callosum, and 3.27 p. m. in the gray substance of the cortex of the cerebrum (when free as possible from white substance). The white is decidedly richer in neurokeratin than the peripheral nerves or the gray substance. According to GRIFFITHS,[5] *neurochitin* replaces neurokeratin in insects and crustacea, the quantity of the first being 10.6–12 p. m.

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[1] Falk, Bioch. Zeitschr., **13**.

[2] Noll, Zeitschr. f. physiol. Chem., **27**; Mott and Halliburton, Philos. Transactions, Ser. B, **191** (1899), and **194** (1901).

[3] Arch. of Neurology, **3**.

[4] Zeitschr. f. Biologie, **26**.

[5] Compt. rend., **115**.

The quantity of mineral constituents in the brain amounts to 2.95–7.08 p. m. according to GEOGHEGAN. He found in 1000 parts of the fresh, moist brain 0.43–1.32 Cl; 0.956–2.016 PO_4; 0.244–0.796 CO_3: 0.102–0.220 SO_4; 0.01–0.098 $Fe_2(PO_4)_2$; 0.005–0.022 Ca; 0.016–0.072 Mg; 0.58–1.778 K; 0.450–1.114 Na. The gray substance yields an alkaline ash, the white an acid ash.

Appendix.

THE TISSUES AND FLUIDS OF THE EYE.

The retina contains in all 865–899.9 p. m. water, 57.1–84.5 p. m. protein bodies—myosin, albumin, and mucin (?), 9.5–28.9 p. m. lecithin, and 8.2–11.2 p. m. salts (HOPPE-SEYLER and CAHN [1]). The mineral bodies consist of 422 p. m. Na_2HPO_4 and 352 p. m. NaCl.

Those bodies which form the different segments of the rods and cones have not been closely studied, and the greatest interest is therefore connected with the coloring matters of the retina.

Visual purple, also called *rhodopsin, erythropsin,* or VISUAL RED, is the pigment of the rods. BOLL,[2] in 1876, observed that the layer of rods in the retina during life had a purplish-red color which was bleached by the action of light. KÜHNE [3] later showed that this red color might remain for a long time after the death of the animal if the eye was protected from daylight or investigated by a sodium light. Under these conditions it was also possible to isolate and closely study this substance.

Visual red (BOLL) or visual purple (KÜHNE) has become known mainly by the investigations of KÜHNE. The pigment chiefly occurs in the rods and only in their outer parts. In animals whose retina has no rods the visual purple is absent, and is also necessarily absent in the macula lutea. In a variety of bat (Rhinolophus hipposideros), in hens, pigeons and newborn rabbits, no visual purple has been found in the rods.

A solution of visual purple in water which contains 2–5 per cent crystallized bile, which is the best solvent for it, is purple-red in color, quite clear, and not fluorescent. On evaporating this solution *in vacuo* we obtain a residue similar to ammonium carminate which contains violet or black grains. If the above solution is dialyzed with water, the bile diffuses and the visual purple separates as a violet mass. Under all circumstances, even when still in the retina, the visual purple is quickly bleached by direct sunlight, and with diffused light with a rapidity corre-

[1] Zeitschr. f. physiol. Chem., **5**.

[2] Monatsschr. d. Kgl. Preuss. Akad., 12. Nov., 1876.

[3] The investigations of Kühne and his pupils, Ewald and Ayres, on the visual purple will be found in Untersuchungen aus dem physiol. Institut der Universität Heidelberg, **1** and **2**, and in Zeitschr. f. Biologie, **32**.

sponding to the intensity of the light. It passes from red and orange to yellow. Red light bleaches the visual purple slowly; the ultra-red light does not bleach it at all. A solution of visual purple shows no special absorption bands, but only a general absorption which extends from the red side, beginning at *D* and extending to the *G* line. The strongest absorption is found at *E*.

KOETTGEN and ABELSDORF [1] have shown that there are, in accordance with KÜHNE'S views, two varieties of visual purple, the one occurring in mammals, birds, and amphibians, and the other, which is more violet-red, in fishes. The first has its maximum absorption in the green and the other in the yellowish green.

Visual purple when heated to 52–53° C. is destroyed after several hours, and almost instantly when heated to 76° C. It is also destroyed by alkalies, acids, alcohol, ether, and chloroform. On the contrary, it resists the action of ammonia or alum solution.

As the visual purple is easily destroyed by light, it must therefore also be regenerated during life. KÜHNE has also found that the retina of the eye of the frog becomes bleached when exposed for a long time to strong sunlight, and that its color gradually returns when the animal is placed in the dark. This regeneration of the visual purple is a function of the living cells in the layer of the pigment epithelium of the retina. This may be inferred from the fact that a detached piece of the retina which has been bleached by light may have its visual purple restored if it is carefully laid on the choroid having layers of the pigment-epithelium attached. The regeneration has, it seems, nothing to do with the dark pigment, the melanin or fuscin, in the epithelium cells. A partial regeneration seems, according to KÜHNE, to be possible in the retina which has been completely removed. On account of this property of the visual purple of being bleached by light during life we may, as KÜHNE has shown, under special conditions and by observing special precautions, obtain after death, by the action of intense light or more continuous light, the picture of bright objects, such as windows and the like—so-called optograms.

The physiological importance of visual purple is unknown. It follows that the visual purple is not essential to sight, since it is absent in certain animals and also in the cones.

Visual purple must always be prepared exclusively in a sodium light. It is extracted from the net membrane by means of a watery solution of crystallized bile. The filtered solution is evaporated *in vacuo* or dialyzed until the visual purple is separated. To prepare a visual-purple solution perfectly free from hæmoglobin the solution of visual purple

[1] Centralbl. f. Physiol., 9; also Maly's Jahresber , 25, 351.

in cholates is precipitated by saturating with magnesium sulphate, washing the precipitate with a saturated solution of magnesium sulphate, and then dissolving in water by the aid of the cholates simultaneously precipitated.[1]

The Pigments of the Cones. In the inner segments of the cones of birds, reptiles, and fishes a small fat-globule of varying color is found. KÜHNE [2] has isolated from this fat a green, a yellow, and a red pigment called respectively *chlorophan, xanthophan,* and *rhodophan.*

The dark pigment of the epithelium-cells of the net membrane, which was formerly called *melanin,* but has since been named *fuscin* by KÜHNE and MAYS,[3] contains iron, dissolves in concentrated caustic alkalies or concentrated sulphuric acid on warming, but, like the melanins in general, has been little studied. The pigment occurring in the pigment-cells of the choroid will be discussed with the melanins in Chapter XVI.

The **vitreous humor** is often considered as a variety of gelatinous tissue. The membrane consists, according to C. MÖRNER, of a gelatin-forming substance. The fluid contains a little proteid and a mucoid, *hyalomucoid,* which was first shown by MÖRNER, and which is precipitated by acetic acid. This contains 12.27 per cent N and 1.19 per cent S. Among the extractives we find a little *urea*—according to PICARD 5 p. m., according to RÄHLMANN 0.64 p. m. PAUTZ [4] found besides some urea *paralactic acid,* and, in confirmation of the claims of CHABBAS, JESNER, and KUHN, also *glucose* in the vitreous humor of oxen. The reaction of the vitreous humor is alkaline, and the quantity of solids amounts to about 9–11 p. m. The quantity of mineral bodies is about 6–9 p. m. and the proteins 0.7 p. m. In regard to the aqueous humor see page 342.

The Crystalline Lens. That substance which forms the capsule of the lens has been investigated by C. MÖRNER. It belongs, according to him, to a special group of proteins, called *membranins.* The membranin bodies are insoluble at the ordinary temperature in water, salt solutions, dilute acids, and alkalies, and, like the mucins, yield a reducing substance on boiling with dilute mineral acids. They contain lead-blackening sulphur. The membranins are colored a very beautiful red by MILLON'S reagent, but give no characteristic reaction with concentrated hydrochloric acid or ADAMKIEWICZ'S reagent. They are dissolved with great difficulty by pepsin-hydrochloric acid or trypsin solution, but are soluble in dilute acids and alkalies in the warmth. Membranin of the capsule

[1] Kühne, Zeitschr. f. Biologie, **32**.

[2] Kühne, Die nichtbeständigen Farben der Netzhaut, Untersuch. aus dem physiol. Institut Heidelberg, **1**, 341.

[3] Kühne, *ibid.*, **2**, 324.

[4] Mörner, Zeitschr. f. physiol. Chem., **18**; Picard, cited from Gamgee, Physiol. Chem., **1**, 454; Rählmann, Maly's Jahresber., **6**; Pautz, Zeitschr. f. Biologie, **31**. A complete review of the literature will also be found here.

of the lens contains 14.10 per cent N and 0.83 per cent S, and is a little less soluble than that from DESCEMET'S membrane.

The chief mass of the solids of the crystalline lens consists of proteins, whose nature has been investigated by C. MÖRNER.[1] Some of these proteins dissolve in dilute salt solution, while others remain insoluble in this solvent.

The Insoluble Protein. The lens fibres consist of a protein substance which is insoluble in water and in salt solution and to which MÖRNER has given the name *albumoid.* It dissolves readily in very dilute acids or alkalies. Its solution in caustic potash of 0.1 per cent is very similar to an alkali-albuminate solution, but coagulates at about 50° C. on nearly complete neutralization and the addition of 8 per cent NaCl. Albumoid has the following composition: C 53.12, H 6.8, N 16.62, and S 0.79 per cent. The lens fibres themselves contain 16.61 per cent N and 0.77 per cent S. The inner parts of the lens are considerably richer in albumoid than the outer. The quantity of albumoid in the entire lens amounts on an average to about 48 per cent of the total weight of the proteins of the lens.

The Soluble Protein consists, exclusive of a very small quantity of *albumin,* of two globulins, *α-* and *β-crystallin.* These two globulins differ from each other in this manner: *α*-crystallin contains 16.68 per cent N and 0.56 per cent S; *β*-crystallin, on the contrary, 17.04 per cent N and 1.27 per cent S. The first coagulates at about 72° C. and the other at 63° C. Besides this, *β*-crystallin is precipitated from a salt-free solution with greater difficulty and less completely by acetic acid or carbon dioxide. These globulins are not precipitated by an excess of NaCl at either the ordinary temperature or 30° C. Magnesium or sodium sulphate in substance precipitates both globulins, on the contrary, at 30° C. These two globulins are not equally divided in the mass of the lens. The quantity of *α*-crystallin diminishes in the lens from without inward; *β*-crystallin, on the contrary, from within outward.

A. BÉCHAMP distinguishes the two following protein bodies in the watery extract of the crystalline lens: *phacozymase,* which coagulates at 55° C., contains a diastatic enzyme, and has a specific rotatory power of $(\alpha)j = -41°$, and the *crystalbumin,* with a specific rotatory power of $(\alpha)j = -80.3°$. From the residue of the lens, which was insoluble in water, BÉCHAMP extracted, by means of hydrochloric acid, a protein body having a specific rotatory power of $(\alpha)j = -80.2°$, which he called *crystalfibrin.*

The lens does not seem to contain any protein bodies which coagulate spontaneously like fibrinogen. That cloudiness which appears after death depends, according to KÜHNE, upon the unequal changing of the concentration of the contents of the lens-tubes. This change is produced

[1] Zeitschr. f. physiol. Chem., 18. This contains also the pertinent literature.

by the altered ratio of diffusion. A cloudiness of the lens may also be produced in life by a rapid removal of water, as, for example, when a frog is plunged into a salt or sugar solution. The appearance of cloudiness in diabetes has been attributed by some to the removal of water. Opinions on this subject are, however, conflicting.

The average results of four analyses made by LAPTSCHINSKY [1] of the lens of oxen are here given, calculated in parts per 1000:

Proteins	349.3
Lecithin	2.3
Cholesterin	2.2
Fat	2.9
Soluble salts	5.3
Insoluble salts	2.4

In cataract the amount of proteins is diminished and the amount of cholesterin increased. This statement requires further substantiation.[2]

The quantity of the different proteins in the fresh moist lens of oxen is as follows, according to MÖRNER [3]:

Albumoid (lens fibres)	170 p. m.
β-Crystallin	110 "
α-Crystallin	68 "
Albumin	2 "

The corneal tissue has been previously considered (page 525). **The sclerotic** has not been closely investigated, and **the choroid coat** is chiefly of interest because of the coloring-matter (melanin) it contains (see Chapter XVI).

TEARS consist of a water-clear, alkaline fluid of a salty taste. According to the analyses of LERCH [4] they contain 982 p. m. water, 18 p. m. solids with 5 p. m. albumin and 13 p. m. NaCl.

THE FLUIDS OF THE INNER EAR.

The perilymph and **endolymph** are alkaline fluids, which, besides salts, contain—in the same amounts as in transudates—traces of *protein*, and in certain animals (codfish) also *mucin*. The quantity of mucin is greater in the perilymph than in the endolymph.

Otoliths contain 745–795 p. m. inorganic substance, which consists chiefly of crystallized calcium carbonate. The organic substance is very similar to mucin.

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[1] Pflüger's Arch., **13**.

[2] See Gross, Arch. f. Augenheilk., **55** and **58**.

[3] l. c.

[4] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 401.

CHAPTER XIII.

ORGANS OF GENERATION.

(a) Male Generative Secretions.

The **testes** have been little investigated chemically. We find in the testes of animals protein bodies of different kinds—*seralbumin, alkali albuminate* (?), and an albuminous body related to ROVIDA'S *hyaline substance*; also *leucine, tyrosine, creatine, purine bases, cholesterin, lecithin, inosite,* and *fat.* In regard to the occurrence of glycogen the reports are conflicting. DARESTE [1] found, in the testes of birds, starch-like granules, which were colored blue with difficulty by iodine.

In the autolysis of the testes LEVENE [2] found tyrosine, alanine, leucine, aminovaleric acid, aminobutyric acid, α-proline, phenylalanine, aspartic acid, glutamic acid, and hypoxanthine. Pyrimidine and hexone bases could not be detected.

The **semen** as ejected is a white or whitish-yellow, viscous, sticky fluid of a milky appearance, with whitish, non-transparent lumps. The milky appearance is due to spermatozoa. Semen is heavier than water, contains proteins, has a neutral or faintly alkaline reaction and a peculiar specific odor. Soon after ejection semen becomes gelatinous, as if it were coagulated, but afterward becomes more fluid. When diluted with water white flakes or shreds separate (HENLE'S *fibrin*). According to the analyses of SLOWTZOFF,[3] human semen contains on an average 96.8 p. m. solids with 9 p. m. inorganic and 87.8 p. m. organic substance. The amount of protein substances was, on an average, 22.6 p. m. and 1.69 p. m. of bodies soluble in ether. The protein substances consist of *nucleoproteins,* traces of *mucin, albumin,* and a substance similar to *proteose* (found earlier by POSNER). According to CAVAZZANI [4] semen contains relatively considerable *nucleon,* more than any organ. The mineral bodies consist chiefly of calcium phosphate and considerable NaCl. Potassium occurs only in smaller amounts.

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[1] Compt. rend., **74.**

[2] Amer. Journ. of Physiol., **11.**

[3] Zeitschr. f. physiol. Chem., **35.**

[4] Posner, Berl. klin. Wochenschr., 1888, No. 21, and Centralbl. f. d. med. Wissensch., 1890; Cavazzani, Biochem. Centralbl., **1**, 502, and Centralbl. f. Physiol., **19.**

The semen in the vas deferens differs chiefly from the ejected semen in that it is without the peculiar odor. This last depends on the admixture with the secretion of the prostate. This secretion, according to IVERSEN, has a milky appearance and ordinarily an alkaline reaction, very rarely a neutral one, and contains small amounts of proteins, especially *nucleoproteins*, besides a substance similar to *fibrinogen* and to *mucin* (STERN [1]), and mineral bodies, especially NaCl. Besides this it contains an enzyme *vesiculase* (see below), *lecithin*, *choline* (STERN), and a crystalline combination of phosphoric acid with a base, C_2H_5N. This combination has been called BÖTTCHER'S *spermine crystals*, and it is claimed that the specific odor of the semen is due to a partial decomposition of these crystals.

The crystals which appear on slowly evaporating the semen, and which are also observed in anatomical preparations kept in alcohol, are not identical with the CHARCOT-LEYDEN crystals found in the blood and in the lymphatic glands in leucæmia (TH. COHN, B. LEWY [2]). They are, according to SCHREINER,[3] as above stated, a combination of phosphoric acid with a base, *spermine*, C_2H_8N, which he discovered.

Spermine. Opinions in regard to the nature of this base are not unanimous. According to the investigations of LADENBURG and ABEL, it is not improbable that spermine is identical with ethylenimine; but this identity is disputed by MAJERT and A. SCHMIDT, and also by POEHL. The compound of spermine with phosphoric acid—BÖTTCHER'S spermine crystals—is insoluble in alcohol, ether, and chloroform, soluble with difficulty in cold water, but more readily in hot water, and easily soluble in dilute acids or alkalies, also alkali carbonates and ammonia. The base is precipitated by tannic acid, mercuric chloride, gold chloride, platinic chloride, potassium-bismuth iodide, and phosphotungstic acid. Spermine has a tonic action, and according to POEHL [4] it has a marked action on the oxidation processes of the animal body.

On the addition of a solution of potassium iodide and iodine to spermatozoa, characteristic dark-brown or bluish-black crystals are obtained—FLORENCE'S sperm reaction, which is considered by many as a reaction for spermine. According to BOCARIUS,[5] this reaction is due to choline.

CAMUS and GLEY [6] have found that the prostate fluid in certain rodents has the property of coagulating the contents of the seminal vesicles. This property is due to a special ferment substance (*vesiculase*) of the prostate fluid.

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[1] Iversen, Nord. med. Ark., **6**; also Maly's Jahresber., **4**, 358; Stern, Biochem. Centralbl., **1**, 748.

[2] Th. Cohn, Centralbl. f. allg. Path. u. path. Anat., **10** (1899), and Zeitschr. f. Urolog., 1908; B. Lewy, Centralbl. f. d. med. Wissensch., 1899, 479.

[3] Annal. d. Chem. u. Pharm., **194**.

[4] Ladenburg and Abel, Ber. d. deutsch. chem. Gesellsch., **21**; Majert and A. Schmidt, *ibid.*, **24**; Poehl, Compt. rend., **115**, Berlin. klin. Wochenschr., 1891 and 1893, Deutsch. med. Wochenschr., 1892 and 1895, and Zeitschr. f. klin. Med., 1894.

[5] In regard to Florence's sperm reaction, see Posner, Berl. klin. Wochenschr., 1897, and Richter, Wien. klin. Wochenschr., 1897; Bocarius, Zeitschr. f. physiol. Chem., **34**.

[6] Compt. rend. de soc. biolog., **48**, **49**.

The **spermatozoa** show a great resistance to chemical reagents in general. They do not dissolve completely in concentrated sulphuric acid, nitric acid, acetic acid, or in boiling-hot soda solutions. They are soluble in a boiling-hot caustic-potash solution. They resist putrefaction, and after drying they may be obtained again in their original form by moistening them with a 1-per cent common-salt solution. By careful heating and burning to an ash the shape of the spermatozoa may be seen in the ash. The quantity of ash is about 50 p. m. and consists mainly (three quarters) of potassium phosphate.

The spermatozoa show well-known movements, but the cause of this is not known. These movements may continue for a very long time, as under some conditions they may be observed for several days in the body after death, and in the secretion of the uterus longer than a week. Acid liquids stop these movements immediately; they are also destroyed by strong alkalies, especially ammoniacal liquids, also by distilled water, alcohol, ether, etc. The movements continue for a longer time in faintly alkaline liquids, especially in alkaline animal secretions, and also in properly diluted neutral salt solutions.[1]

Spermatozoa are nucleus formations and hence are rich in nucleic acid, which exists in the heads. The tails contain protein and are besides this rich in lecithin, cholesterin, and fat, which bodies occur only to a small extent (if at all) in the heads. The tails seem by their composition to be closely allied to the non-medullated nerves or the axis-cylinders. In the various kinds of animals investigated, the head contains nucleic acid, which in fishes is partly combined with protamines and partly with histones. In other animals, such as the bull and boar, protein-like substances occur with the nucleic acid, but no protamine.

Our knowledge of the chemical composition of spermatozoa has been greatly enhanced by the important investigations of Miescher[2] on salmon milt. The intermediate fluid of the spermatozoa of Rhine salmon is a dilute salt solution containing 1.3–1.9 p. m. organic and 6.5–7.5 p. m. inorganic bodies. The last consist chiefly of sodium chloride and carbonate, besides some potassium chloride and sulphate. The fluid contains only traces of protein, but no peptone. The tails consist of 419 p. m. protein, 318.3 p. m. lecithin, and 262.7 p. m. cholesterin and fat. The heads extracted with alcohol-ether contain on an average 960 p. m. protamine nucleate, which nevertheless is not uniform, but is so divided that the outer layers consist of basic protamine nucleate, while the inner layers, on the contrary, consist of acid protamine nucleate. Besides the protamine nucleate there are present in the heads, although

[1] See G. Günther, Pflüger's Arch., 118.

[2] See Miescher, "Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, gesammelt und herausgegeben von seinen Freunden," Leipzig, 1897.

to a very slight extent, organic substances. Of these we must mention a nitrogenous substance containing iron which gives MILLON'S reaction and which MIESCHER calls *karyogen*. The unripe salmon spermatozoa, while developing, also contain nucleic acid, but no protamine, with a protein substance, "*albuminose*," which probably is a step in the formation of protamine. According to KOSSEL and MATHEWS,[1] in the herring as in the salmon, the heads of the spermatozoa consist of protamine nucleate but no free protein.

The chemical investigations on the spermatozoa have not given us any information as to the condition for fertilization and the development of the egg.

Spermatin is a name which has been given to a constituent similar to alkali albuminate, but it has not been closely studied.

Prostatic concrements are of two kinds. One is very small, generally oval in shape, with concentric layers. In young but not in older persons they are colored blue by iodine (IVERSEN [2]). The other kind is larger, sometimes the size of the head of a pin, and consisting chiefly of calcium phosphate (about 700 p. m.), with only a very small amount (about 160 p. m.) of organic substance.

(b) Female Generative Organs.

The stroma of the **ovaries** is of little interest from a physiologico-chemical standpoint, and the most important constituents of the ovaries, the *Graafian follicles* with the *ovum*, have not thus far been the subject of a careful chemical investigation. The fluid in the follicles (of the cow) does not contain, as has been stated, the peculiar bodies, paralbumin or metalbumin, which are found in certain pathological ovarial fluids, but seems to be a serous liquid. The *corpora lutea* are colored yellow by an amorphous pigment called *lutein*. Besides this another coloring-matter sometimes occurs which is not soluble in alkali; it is crystalline, but not identical with bilirubin or hæmatoidin; but it may be identified as a lutein by its spectroscopic behavior (PICCOLO and LIEBEN, KÜHNE and EWALD [3]).

The cysts often occurring in the ovaries are of special pathological interest, and these may have essentially different contents, depending upon their variety and origin.

The **serous cysts** (HYDROPS FOLLICULORUM GRAAFII), which are formed by a dilation of the Graafian follicles, contain a serous liquid which has a specific gravity of 1.005–1.022. A specific gravity of 1.020 is less frequent. Generally the specific gravity is lower, 1.005–1.014, with 10–40 p. m. solids. As far as is known, the contents of these cysts do not essentially differ from other serous liquids.

[1] Zeitschr. f. physiol. Chem., **23**.

[2] Nord. med. Ark., **6**.

[3] See Chapter VI, p. 290.

The proliferous cysts (MYXOID CYSTS, COLLOID CYSTS), which are developed from PFLÜGER'S epithelium-tubes, may have a content of a decidedly variable composition.

We sometimes find in small cysts a semi-solid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called *colloid* because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and as this mass in the different cysts is more or less diluted with serous liquids their contents may have a variable consistency. In still other cases the small cysts may also contain a thin, watery fluid. The color of the contents is also variable. Sometimes they are bluish white, opalescent, and again they are yellow, yellowish brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or red-brown, due to the decomposed blood-coloring matters. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1.015–1.030, but may occasionally be 1.005–1.010 or 1.050–1.055. The amount of solids is very variable. In rare cases it amounts to only 10–20 p. m.; ordinarily it varies between 50–70–100 p. m. In a few instances 150–200 p. m. solids have been found.

As form-elements one finds red and white *blood-corpuscles*, *granular cells*, partly fat-degenerated epithelium and partly large so-called GLUGE'S corpuscles, *fine granular masses*, *epithelium-cells*, *cholesterin crystals*, and *colloid corpuscles*—large, circular, highly refractive formations.

Though the contents of the proliferous cyst may have a variable composition, still it may be characterized in typical cases by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity, 1.015–1.025. Such a liquid does not ordinarily show a spontaneous fibrin coagulation.

We consider *colloid*, *metalbumin*, and *paralbumin* as characteristic constituents of these cysts.

Colloid. This name does not designate any particular chemical substance, but is given to the contents of tumors with certain physical properties similar to gelatin jelly. Colloid is found as a pathological product in several organs.

Colloid is a gelatinous mass, insoluble in water and acetic acid; it is dissolved by alkalies and gives a liquid which is not precipitated by acetic acid or by acetic acid and potassium ferrocyanide. According to PFANNENSTIEL [1] such a colloid is designated β-pseudomucin. Sometimes a colloid is found which, when treated with a very dilute alkali,

[1] Arch. f. Gynäk., 38.

gives a solution similar to a mucin solution. Colloid is very closely related to mucin and is considered by certain investigators as a modified mucin. An ovarial colloid analyzed by PANZER contained 931 p. m. water, 57 p. m. organic substance, and 12 p. m. ash. The elementary composition was C 47.27, H 5.86, N 8.40, S 0.79, P 0.54, and ash 6.43 per cent. A colloid found by WÜRTZ [1] in the lungs contained C 48.09, H 7.47, N 7.00, and O(+S) 37.44 per cent. Colloids of different origin seem to be of varying composition.

Metalbumin. This name SCHERER [2] gave to a protein substance found by him in an ovarial fluid. The metalbumin was considered by SCHERER to be an albuminous body, but it belongs to the mucin group, and it is for this reason called *pseudomucin* by HAMMARSTEN.[3]

Pseudomucin. This body, which, like the mucins, gives a reducing substance when boiled with acids, is a mucoid of the following composition: C 49.75, H 6.98, N 10.28, S 1.25, O 31.74 per cent (HAMMARSTEN). With water pseudomucin gives a slimy, ropy solution, and it is this substance which gives the fluid contents of the ovarial cysts their typical ropy property. Its solutions do not coagulate on boiling, but only become milky or opalescent. Unlike mucin, pseudomucin solutions are not precipitated by acetic acid. With alcohol they give a coarse flocculent or thready precipitate which is soluble even after having been kept under water or alcohol for a long time.

Paralbumin is another substance discovered by SCHERER, which occurs in ovarial liquids and also in ascitic fluids with the simultaneous presence of ovarial cysts and rupture of the same. It is therefore only a mixture of pseudomucin with variable amounts of protein, and the reactions of paralbumin are correspondingly variable.

MITJUKOFF [4] has isolated and investigated a colloid from an ovarial cyst. It had the following composition: C 51.76, H 7.76, N 10.7, S 1.09, and O 28.69 per cent, and differed from mucin and pseudomucin by reducing FEHLING'S solution before boiling with acid. It must be remarked that pseudomucin, on boiling sufficiently long with alkali, or by the use of a concentrated solution of caustic alkali, also splits and causes a reduction. This reduction is nevertheless weak as compared with that produced after boiling with an acid. The body isolated by MITJUKOFF is called *paramucin.*

The pseudomucin as well as colloid are mucoid substances, and the carbohydrate obtained from them is glucosamine (chitosamine), as espe-

[1] Panzer, Zeitschr. f. physiol. Chem., 28; Würtz, see Lebert, Beitr. zur Kenntnis des Gallertkrebses, Virchow's Arch., 4.

[2] Verh. d. physik.-med. Gesellsch. in Würzburg, 2, and Sitzungsber. der physik.-med. Gesellsch. in Würzburg für 1864–1865; Würzburg med. Zeitschr., 7, No. 6.

[3] Zeitschr. f. physiol. Chem., 6.

[4] K. Mitjukoff, Arch. f. Gynäkol., 49.

cially shown by Fr. Müller, Neuberg and Heymann.[1] From pseudomucin Zängerle [2] obtained 30 per cent glucosamine, and Neuberg and Heymann have shown that the glucosamine is the only carbohydrate regularly taking part in the structure of these substances. Still there are reports as to the occurrence of chondroitin-sulphuric acid (or an allied acid) in pseudomucin or colloid (Panzer), but this is not constant according to the experience of Hammarsten.

As hydrolytic cleavage products of pseudomucin Otori obtained, besides carbohydrate derivatives such as levulinic acid and humus substances, leucine, tyrosine, glycocoll, aspartic acid, glutamic acid, valeric acid, arginine, lysine, and guanidine. The quantity of guanidine, it seems, was greater than that which could be derived from the arginine, hence this body probably originated from another complex. Pregl [3] obtained on the hydrolysis of a colloid, which behaved like paramucin, no glycocoll and only traces of diamino acids, but otherwise the same amino acids as Otori found, besides alanine, proline, phenylalanine and tryptophane.

The detection of metalbumin and paralbumin is naturally connected with the detection of pseudomucin. A typical ovarial fluid containing pseudomucin is, as a rule, sufficiently characterized by its physical properties, and a special chemical investigation is necessary only in cases where a serous fluid contains very small amounts of pseudomucin. The procedure is as follows: The protein is removed by heating to boiling with the addition of acetic acid; the filtrate is strongly concentrated and precipitated by alcohol. The precipitate, a transformation product of pseudomucin, is carefully washed with alcohol and then dissolved in water. A part of this solution is digested with saliva at the temperature of the body and then tested for glucose (derived from glycogen or dextrin). If glycogen is present, it will be converted into glucose by the saliva; precipitate again with alcohol and then proceed as in the absence of glycogen. In this last-mentioned case, first add acetic acid to the solution of the alcohol precipitate in water so as to precipitate any existing mucin. The precipitate produced is filtered off, the filtrate treated with 2-per cent HCl and warmed on the water-bath until the liquid is deep brown in color. In the presence of pseudomucin this solution gives Trommer's test.

The other protein bodies which have been found in cystic fluids are *serglobulin* and *seralbumin*, *peptone* (?), *mucin*, and *mucin-peptone* (?). Fibrin occurs only in exceptional cases. The quantity of mineral bodies on an average amounts to about 10 p. m. The amount of extractive bodies (*cholesterin* and *urea*) and *fat* is ordinarily 2–4 p. m. The remaining

[1] Müller, Verh. d. Naturf. Gesellsch. in Basel, **12**, part 2; Neuberg and Heymann, Hofmeister's Beiträge, **2**. See also Leathes, Arch. f. exp. Path. u. Pharm., **43**.

[2] Münch. med. Wochenschr., 1900.

[3] Otori, Zeitschr. f. physiol. Chem., **42** and **43**; Pregl, *ibid.*, **58**.

solids, which constitute the chief mass, are protein bodies and pseudomucin.

The **intraligamentary, papillary cysts** contain a yellow, yellowish-green, or brownish-green liquid which contains either no pseudomucin or very little. The specific gravity is generally rather high, 1.032–1.036, with 90–100 p. m. solids. The principal constituents are the simple proteins of blood-serum.

The rare *tubo-ovarial cysts* contain as a rule a watery, serous fluid containing no pseudomucin.

The *parovarial cysts* or the CYSTS of the LIGAMENTA LATA may attain a considerable size. In general, and when quite typical, the contents are watery, mostly very pale-yellow-colored, water-clear or only slightly opalescent liquids. The specific gravity is low, 1.002–1.009, and the solids only amount to 10–20 p. m. Pseudomucin does not occur as a typical constituent; protein is sometimes absent, and when it does occur the quantity is very small. The principal part of the solids consists of salts and extractive bodies. In exceptional cases the fluid may be rich in protein and may show a higher specific gravity.

In regard to the quantitative composition of the fluid from ovarial cysts we refer the reader to the work of OERUM.[1]

E. LUDWIG and R. v. ZEYNEK have investigated the fat from dermoid cysts. Besides a little arachidic acid, they found oleic, stearic, palmitic, and myristic acids, cetyl alcohol, and a cholesterin-like substance. In regard to the occurrence of cetyl alcohol see the work of AMESEDER,[2] page 232.

The colloid from a uterine fibroma analyzed by STOLLMANN [3] contained a pseudomucin soluble in water and a colloid (paramucin) insoluble in water, both of which behaved differently with alcohol as compared with the corresponding substances from ovarial cysts.

The Ovum.

The small ova of man and mammals cannot, for evident reasons, be the subject of a searching chemical investigation. Up to the present time the eggs of birds, amphibians, and fishes have been investigated, but above all the hen's egg. We will here occupy ourselves with the constituents of this last.

The Yolk of the Hen's Egg. In the so-called white yolk, which forms the *germ* with a process reaching to the center of the yolk (*latebra*), and forming a layer between the yolk and yolk-membrane, there occurs *protein, nuclein, lecithin,* and *potassium* (LIEBERMANN [4]). The occur-

[1] Kemiske Studier over Ovariecystevædsker, etc., Koebenhavn, 1884. See also Maly's Jahresber., **14**, 459.

[2] Ludwig and v. Zeynek, Zeitschr. f. physiol. Chem., **23**; Ameseder, *ibid.*, **52**.

[3] Amer. Gynecology, 1903.

[4] Pflüger's Arch., **43**.

rence of glycogen is doubtful. The yolk-membrane consists of an albuminoid similar in certain respects to keratin (LIEBERMANN).

The principal part of the yolk—the nutritive yolk or yellow—is a viscous, non-transparent, pale-yellow or orange-yellow alkaline emulsion of a mild taste. The yolk contains *vitellin, lecithin, cholesterin, fat, coloring-matters,* traces of *neuridine* (BRIEGER [1]), *purine bases* (MESERNITZKI [2]), *glucose* in very small quantities, and *mineral bodies.* The occurrence of cerebrin and of granules similar to starch (DARESTE [3]) has not been positively proven.

Several enzymes have been found in the yolk, especially a diastatic enzyme (MÜLLER and MASUYAMA), a glycolytic enzyme (STEPANEK) which in the absence of air brings about an alcoholic fermentation of sugar and in the presence of air forms carbon dioxide and lactic acid, and finally a proteolytic, a lipolytic, and a chromolytic (?) enzyme (WOHLGEMUTH[4]).

Ovovitellin. This body, which is often considered as a globulin, is in reality a nucleoalbumin. The question as to what relation other protein substances which are related to ovovitellin, like the *aleuron grains* of certain seeds and the *yolk spherules* of the eggs of certain fishes and amphibians, bear to this substance is one which requires further investigation.

The ovovitellin which has been prepared from the yolk of eggs is not a pure protein body, but always contains lecithin. HOPPE-SEYLER found 25 per cent lecithin in vitellin. The lecithin may be removed by boiling alcohol, but the vitellin is changed thereby, and it is therefore probable that the lecithin is chemically united with the vitellin (HOPPE-SEYLER [5]). According to OSBORNE and CAMPBELL, the so-called ovovitellin is a mixture of various vitellin-lecithin combinations, with 15 to 30 per cent of lecithin. The protein substance freed from lecithin is the same in all these compounds and has the following composition: C 51.24, H 7.16, N 16.38, S 1.04, P 0.94, O 23.24 per cent. These figures differ somewhat from those obtained by GROSS for vitellin prepared by another method (precipitation with $[NH_4]_2SO_4$), namely, C 48.01, H 6.35, N 14.91–16.97, P 0.32–0.35, S 0.88, and the composition of ovovitellin is therefore not positively known. Besides the vitellin GROSS found a globulin coagulating at 76–77° C. in a solution containing salt, and PLIMMER [6] found a protein

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[1] Ueber Ptomaïne, Berlin, 1885.

[2] Mesernitzki, Biochem. Centralbl., **1**, 739.

[3] Compt. rend., **72**.

[4] Müller and Masuyama, Zeitschr. f. Biologie, **39**; Stepanek, Centralbl. f. Physiol., **18**, 188; Wohlgemuth in Salkowski's Festschrift and Zeitschr. f. physiol. Chem., **44**.

[5] Med. chem. Untersuch., 216.

[6] Osborne and Campbell, Connecticut Agric. Exp. Station, 23d Ann. Report, New

which he calls *livetin* which only contained 0.1 per cent phosphorus and which gave more monamino acids but less amide and diamino nitrogen than vitellin.

On the pepsin digestion of ovovitellin, OSBORNE and CAMPBELL obtained a pseudonuclein with varying amounts of phosphorus, 2.52–4.19 per cent. BUNGE [1] prepared a pseudonuclein by digesting the yolk with gastric juice, and his pseudonuclein, he claims, is of great importance in the formation of the blood, and on these grounds he called it *hæmatogen*. This hæmatogen has the following composition: C 42.11, H 6.08, N 14.73, S 0.55, P 5.19, Fe 0.29, and O 31.05 per cent. The composition of this substance may vary considerably even on using the same method of preparation.

Vitellin is similar to the globulins in that it is insoluble in water, but on the contrary soluble in dilute neutral-salt solutions (although the solution is not quite transparent). It is also soluble in hydrochloric acid of 1 p. m. and in very dilute solutions of alkalies or alkali carbonates. It is precipitated from its salt solution by diluting with water, and when allowed to stand some time in contact with water the vitellin is gradually changed, forming a substance more like the albuminates. The coagulation temperature for the solution containing salt (NaCl) lies between 70° and 75° C., or, when heated very rapidly, at about 80° C. Vitellin differs from the globulins in yielding pseudonuclein by peptic digestion. It is not always completely precipitated by NaCl in substance. The ovovitellin isolated by GROSS gave MOLISCH'S reaction. NEUBERG [2] has also split off glucosamine from the yolk and has identified it as norisosaccharic acid. It is difficult to state whether this glucosamine was derived from the vitellin or from some other constituent of the yolk.

The chief points in the preparation of ovovitellin are as follows: The yolk is thoroughly agitated with ether; the residue is dissolved in a 10-per cent common-salt solution, filtered, and the vitellin precipitated by adding an abundance of water. The vitellin is now purified by repeatedly redissolving in dilute common-salt solutions and precipitating with water.

Ichthulin, which occurs in the eggs of the carp and other fishes is, according to KOSSEL and WALTER, an amorphous modification of the crystalline body *ichthidin*, which occurs in the eggs of the carp. Ichthulin is precipitated on diluting with water. It was formerly considered as a vitellin. According to WALTER it yields a pseudonuclein on peptic digestion; and this pseudonuclein gives a reducing carbohydrate on boiling with sulphuric acid. Ichthulin has

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Haven, 1900; Gross, Zur Kenntn. d. Ovovitellins, Inaug.-Diss. Strassburg, 1899; Plimmer, Journ. Chem. Soc. London, **93**.

[1] Zeitschr. f. physiol. Chem., **9**, 49. See also Hugounenq and Morel, Compt. rend., **140** and **141**.

[2] Ber. d. d. chem. Gesellsch., **34**.

the following composition: C 53.42, H 7.63, N 15.63, O 22.19, S 0.41, P 0.43 per cent. It also contains iron. The ichthulin investigated by LEVENE from codfish eggs had the composition C 52.44, H 7.45, N 15.96, S 0.92, P 0.65, Fe+O 22.58 per cent, and yielded no reducing substances on boiling with acids. The pure vitellin isolated by HAMMARSTEN [1] from perch eggs had a similar behavior and was very readily changed by a little hydrochloric acid so that it was converted into a typical pseudonuclein. The codfish ichthulin yielded a pseudonucleic acid with 10.34 per cent phosphorus, but this acid still gave the protein reactions.

The yolk also contains *albumin*, besides vitellin and the above-mentioned proteins.

The *fat* of the yolk of the egg, LIEBERMANN [2] claims, is a mixture of a solid and a liquid fat. The solid fat consists chiefly of tripalmitin with some tristearin. On the saponification of the egg-oil LIEBERMANN obtained 40 per cent oleic acid, 38.04 per cent palmitic acid, and 15.21 per cent stearic acid. The fat of the yolk of the egg contains less carbon than other fats, which may depend upon the presence of monoglycerides and diglycerides, or upon a quantity of fatty acid deficient in carbon (LIEBERMANN). The composition of yolk fat is dependent upon the food, as HENRIQUES and HANSEN [3] have shown that the fat of the food passes into the egg.

The phosphatides of the yolk seem to be of various kinds. THIERFELDER and STERN have found three different phosphatides. One of these, which was soluble in alcohol-ether, behaved like lecithin. The second was difficultly soluble in alcohol but readily soluble in ether, contained 1.37 per cent N and 3.96 per cent P. The third was a diamido phosphatide soluble with difficulty in ether but obtained in crystalline needles from hot alcohol and contained 2.77 per cent N and 3.22 per cent P and had a melting point of 160–170° C. FRÄNKEL and BOLAFFIO [4] also found a substance crystallizing from hot alcohol and insoluble in ether with 2.78 per cent N and 2.18 per cent P. They call this body *neottin* and claim that it is a triamido-monophosphatide having the formula $C_{84}H_{172}N_3PO_{15}$. BARBIERI has obtained a sulphurized phosphatide called *ovin*, containing 1.35 per cent P, 3.66 per cent N and 0.4 per cent S. The relation of all these bodies to each other must be further studied.

Lutein. Yellow or orange-red amorphous coloring-matters occur in the yellow of the egg and in several other places in the animal organism; for instance, in the blood-serum and serous fluids, fatty tissues, milk-fat, *corpora lutea*, and in the fat-globules of the retina. These coloring-

[1] Walter, Zeitschr. f. physiol. Chem., **15**; Levene, *ibid.*, **32**; Hammarsten, Skand. Arch. f. Physiol., **17**.

[2] Pflüger's Arch., **43**.

[3] Skand. Arch. f. Physiol., **14**.

[4] Thierfelder and Stern, Zeitschr. f. physiol. Chem., **53**; Fränkel and Bolaffio, Bioch. Zeitschr., **9**; Barbieri, Compt. rend., **145**.

matters, which also occur in the vegetable kingdom (THUDICHUM), and whose relation to the vegetable pigments, the xanthophyll group, has recently been shown by SCHUNCK,[1] have been called *luteins* or *lipochromes*.

The luteins, which among themselves show somewhat different properties, are all soluble in alcohol, ether, and chloroform. They differ from the bile-pigment, bilirubin, in that they are not separated from their solution in chloroform by water containing alkali, and also in that they do not give the characteristic play of colors with nitric acid containing a little nitrous acid, but give a transient blue color, and, lastly, they ordinarily show an absorption-spectrum of two bands, of which one covers the line *F* and the other lies between the lines *F* and *G*. LEWIN, MIETHE and STENGER[2] have given exact reports as to the absorption behavior of the lutein from egg-yolk in various solvents. The luteins withstand the action of alkalies so that they are not changed when we remove the fats present by means of saponification.

Lutein has not been prepared pure. MALY[3] found two pigments free from iron in the eggs of a water-spider (Maja squinado)—one a red (*vitellorubin*) and the other a yellow pigment (*vitellolutein*). Both of these pigments are colored blue by nitric acid containing nitrous acid and beautifully green by concentrated sulphuric acid. The absorption-bands, especially of the vitellolutein, correspond very nearly to those of ovolutein.

The *mineral bodies* of the yolk of the egg consist, according to POLÉÇK,[4] of 51.2–65.7 parts soda, 80.5–89.3 potash, 122.1–132.8 lime, 20.7–21.1 magnesia, 11.90–14.5 iron oxide, 638.1–667.0 phosphoric acid, and 5.5–14.0 parts silicic acid in 1000 parts of the ash. We find phosphoric acid and lime the most abundant, and then potash, which is somewhat greater in quantity than the soda. These results are not, however, quite correct: first, because no dissolved phosphate occurs in the yolk (LIEBERMANN), and secondly, in burning, phosphoric and sulphuric acids are produced, and these drive away the chlorine, which is not accounted for in the preceding analyses.

The yolk of the hen's egg weighs about 12–18 grams. The quantity of water and solids amounts, according to PARKE,[5] to 471.9 p. m. and 528.1 p. m. respectively. Among the solids he found 156.3 p. m. protein, 3.53 p. m. soluble and 6.12 p. m. insoluble salts. The quantity of fat, according to PARKE, is 228.4 p. m.; the lecithin, calculated from the

[1] Thudichum, Centralbl. f. d. med. Wissensch., 1869; Schunck, see Chem. Centralbl., 1903, **2**, 1195.

[2] Pflüger's Arch., **124**.

[3] Monatshefte f. Chem. **2**.

[4] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 740.

[5] Hoppe-Seyler, Med. chem. Untersuch., Heft 2, 209.

amount of phosphorus in the organic substance of the alcohol-ether extract, was 107.2 p. m. and the cholesterin 17.5 p. m.

The **white of the egg** is a faintly yellow albuminous fluid inclosed in a framework of thin membranes; and this fluid is in itself very liquid, but seems viscous because of the presence of these fine membranes. That substance which forms the membranes, and of which the *chalaza* consists, seems to be a body closely related to horn substances (LIEBERMANN).

The white of the egg has a specific gravity of 1.045 and always has an alkaline reaction toward litmus. It contains 850–880 p. m. water, 100–130 p. m. protein bodies, and 7 p. m. salts. Among the extractive bodies LEHMANN found a fermentable *variety of sugar* which amounted to 5 p. m. or, according to MEISSNER, 80 p. m. of the solids.[1] Besides these one finds in the white of the egg traces of fats, soaps, lecithin and cholesterin.

The white of the egg of the Insessores becomes transparent on boiling and acts in many respects like alkali albuminate. This albumin TARCHANOFF [2] called "*tatalbumin.*"

The protein substances of the white of egg behave like glycoproteins, as they all yield glucosamine. For the globulin and albumin it has not been proven nor is it probable that the glucosamine belongs to the protein molecule. According to the solution and precipitation properties they are similar to the globulins, albumins or proteoses. The representatives of the first two groups, which until recently were considered as true proteins, are *ovoglobulin* and *ovalbumin.* The proteose-like body is *ovomucoid.*

Ovoglobulin separates in part on diluting the egg-white with water. It is precipitated upon saturation with magnesium sulphate or upon one-half saturation with ammonium sulphate and coagulates at about 75° C. By repeated solution in water and precipitation with ammonium sulphate a part of the globulin becomes insoluble (LANGSTEIN). This also occurs on precipitation by diluting with water or by dialysis, and it is quite possible that the globulin is a mixture. That portion which readily becomes insoluble seems to be identical with EICHHOLZ's glycoprotein or OSBORNE and CAMPBELL's ovomucin. LANGSTEIN obtained 11 per cent of glucosamine from the soluble ovoglobulin. The total quantity of globulins, according to DILLNER, is about 6.7 per cent of the total protein substances, and this corresponds with the recent determinations of OSBORNE and CAMPBELL. In regard to the probable occur-

[1] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 739.
[2] Pflüger's Arch., **31, 33,** and **39.**

rence of several globulins in the white of the egg there are the determinations of CORIN and BERARD as well as of LANGSTEIN,[1] but they have not led to any positive conclusions.

Ovalbumin. The so-called albumin of the egg-white is undoubtedly a mixture of at least two albumin-like proteins. Opinions differ considerably in regard to the number of these proteins (BONDZYNSKI and ZOJA, GAUTIER, BÉCHAMP, CORIN and BERARD, PANORMOFF, and others). Since HOFMEISTER has been able to prepare ovalbumin in a crystalline form, and since HOPKINS and PINKUS[2] have shown that not more than one-half of the ovalbumin can be obtained in such a form, OSBORNE and CAMPBELL have isolated two different ovalbumins or chief fractions; the crystallizable they call *ovalbumin* and the non-crystallizable *conalbumin*. The two fractions have only a slight variation in elementary composition; the conalbumin coagulates between 50–60° C., nearer to 60° C., and the ovalbumin at 64° C. or at a higher temperature. There are no conclusive investigations as to whether the non-crystallizable conalbumin is a mixture or not, and the question concerning the unity of the crystallizable ovalbumin is also disputed. According to BONDZYNSKI and ZOJA, crystallizable ovalbumin is a mixture of several albumins having somewhat different coagulation temperatures, solubilities, and specific rotations, while HOFMEISTER and LANGSTEIN on the contrary believe that crystallizable ovalbumin is a unit. The reports as to the specific rotation of the different fractions unfortunately differ, and the elementary analyses have also given no positive results, as a variation of 1.2–1.7 per cent has been observed in the quantity of sulphur. According to the consistent analyses of OSBORNE and CAMPBELL and of LANGSTEIN, the conalbumin contains about 1.7 per cent sulphur and about 16 per cent nitrogen, while the ovalbumin contains on an average about 15.3 per cent nitrogen. LANGSTEIN[3] obtained 10–11 per cent glucosamine from ovalbumin and about 9 per cent from conalbumin. The ovalbumin, like the conalbumin, has the properties of the albumins in general, but differs from seralbumin in the following: The specific rotation is lower. It is quickly made insoluble by alcohol and is precipitated by a sufficient quantity of HCl, but dissolves in an excess of acid with greater difficulty than the seralbumin. The products isolated by ABDER-

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[1] Langstein, Hofmeister's Beiträge, **1**; Eichholz, Journ. of Physiol., **23**; Osborne and Campbell, Connecticut Agric. Exp. Station, 23d Ann. Report, New Haven, 1900; Dillner, Maly's Jahresber., **15**; Corin and Berard, *ibid.*, 18.

[2] Hofmeister, Zeitschr. f. physiol. Chem., **14**, **16**, and **24**; Gabriel, *ibid.*, **15**; Bondzynski and Zoja, *ibid.*, **19**; Gautier, Bull. Soc. chim., **14**; Béchamp, *ibid.*, **21**; Corin and Berard, l. c.; Hopkins and Pinkus, Ber. d. d. chem. Gesellsch., **31**, and Journ. of Physiol., **23**; Osborne and Campbell, l. c.; Panormoff, Maly's Jahresber., **27** and **28**.

[3] Zeitschr. f. physiol. Chem., **31**.

HALDEN and PREGL [1] on the hydrolysis of ovalbumin do not show anything of special interest.

As in the past certain doubts have existed as to the purity and chemical unity of the ovalbumins or also of the crystalline ovalbumin, so now this doubt has become still stronger since ovalbumin has been prepared partly free from phosphorus and partly with a variable phosphorus content of 0.1–3.06 per cent (KAAS, WILLCOCK and HARDY [2]).

In preparing crystalline ovalbumin, mix, according to HOFMEISTER, the beaten white of egg free from foam with an equal volume of a saturated ammonium-sulphate solution, filter off the globulin, and allow the filtrate to slowly evaporate in thin layers at the temperature of the room. After a time the masses which separate out are dissolved in water, treated with ammonium sulphate-solution until they begin to get cloudy, and allowed to stand. After repeated recrystallization the mass is either treated with alcohol, which makes the crystals insoluble, or they are dissolved in water and purified by dialysis. From these solutions the proteid does not crystallize again on spontaneous evaporation. (See also page 602, foot-note 2, for the HOPKINS and PINKUS method.) WILLCOCK [3] has recently found that magnesium sulphate can also be used in the crystallization of ovalbumin.

Conalbumin can be removed from the filtrate, after the complete crystallization of the ovalbumin, by removing the sulphate by means of dialysis and coagulating by heat.

GAUTIER [4] found a fibrinogen-like substance in the white of the egg, which was changed into a fibrin-like body by the action of a ferment.

Ovomucoid. This substance, first observed by NEUMEISTER and considered by him as a pseudopeptone and then later studied by SALKOWSKI, is, according to C. TH. MÖRNER,[5] a mucoid with 12.65 per cent nitrogen and 2.20 per cent sulphur. Ovomucoid exists in hen's eggs to the extent of about 10 per cent of the total solids.

A solution of ovomucoid is not precipitated by mineral acids nor by organic acids, with the exception of phosphotungstic acid and tannic acid. It is not precipitated by metallic salts, but basic lead acetate and ammonia render it insoluble. Ovomucoid is thrown down by alcohol, but sodium chloride, sodium sulphate, and magnesium sulphate give no precipitates either at the ordinary temperature or when the salts are added to saturation at 30° C. Its solutions are not precipitated by an

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[1] Zeitschr. f. physiol. Chem., **46**.

[2] Kaas, Monatsh. f. Chem.. **27**; Willcock and Hardy, cited from Chem. Centralbl., 1907, **2**, 821.

[3] Journ. of Physiol., **37**.

[4] Compt. rend., **135**.

[5] R. Neumeister, Zeitschr. f. Biologie, **27**; Salkowski, Centralbl. f. d. med. Wissensch., 1893, 513 and 706; C. Mörner, Zeitschr. f. physiol. Chem., **18**. See also Langstein, Hofmeister's Beiträge, **3** (literature).

equal volume of a saturated solution of ammonium sulphate, but are precipitated on adding more salt thereto. The substance is not precipitated on boiling, but the part which has become insoluble in cold water and which has been dried, is dissolved by boiling water. ZANETTI has prepared glucosamine on splitting ovomucoid with concentrated hydrochloric acid, and SEEMANN found that the quantity of glucosamine in ovomucoid was 34.9 per cent.[1]

Ovomucoid may be prepared by removing all the proteins by boiling with the addition of acetic acid and then concentrating the filtrate and precipitating with alcohol. The substance is purified by repeated solution in water and precipitation with alcohol.

PANORMOW believes that the eggs of other birds, such as the pigeon and ducks, contain a special protein in the egg-white, which is not identical with that of the hen's egg. WORM [2] has prepared a crystalline albumin from white of the turkey eggs which contained 15.37 per cent N, 1.6 per cent S and had a specific rotation of $(\alpha)_D = -34.9°$.

The *mineral bodies* of the white of the egg have been analyzed by POLECK and WEBER.[3] They found in 1000 parts of the ash: 276.6–284.5 grams potash, 235.6–329.3 soda, 17.4–29 lime, 17–31.7 magnesia, 4.4–5.5 iron oxide, 238.4–285.6 chlorine, 31.6–48.3 phosphoric acid (P_2O_5), 13.2–26.3 sulphuric acid, 2.8–20.4 silicic acid, and 96.7–116 grams carbon dioxide. Traces of fluorine have also been found (NICKLÉS [4]). The white of egg contains, as compared with the yolk, a greater amount of chlorine and alkalies and a smaller amount of lime, phosphoric acid, and iron.

The Shell-membrane and the Egg-shell. The shell-membrane consists, as above stated (page 112), of a keratin substance. The shell contains very little organic substance, 36–65 p. m. The chief mass, more than 900 p. m., consists of calcium carbonate; besides this there are very small amounts of magnesium carbonate and earthy phosphates.

The diverse *coloring* of bird's eggs is due to several different coloring-matters. Among these we find a red or reddish-brown pigment called "*oorodein*" by SORBY,[5] which is perhaps identical with hæmatoporphyrin. The green or blue coloring-matter, SORBY's *oocyan*, seems, according to LIEBERMANN [6] and KRUKENBERG,[7] to be partly *biliverdin* and partly a blue *derivative of the bile-pigments.*

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[1] Zanetti, Chem. Centralbl., 1898, **1**; Seemann, cited from Langstein, Ergebnisse der Physiol., **1**, Abt. **1**, 86.

[2] Panormow, see Bioch. Centralbl., **5**; Worm, cited from Chem. Centralbl., 1906, **2**, 1508.

[3] Cited from Hoppe-Seyler, Physiol. Chem., 778.

[4] Compt. rend., **43**.

[5] Cited from Krukenberg, Verh. d. phys.-chem. Gesellsch. in Würzburg, **17**.

[6] Ber. d. deutsch. chem. Gesellsch., **11**.

[7] l. c.

The eggs of birds have a space at their blunt end filled with gas; this gas contains on an average 18.0–19.9 per cent oxygen (HÜFNER).[1]

The weight of a hen's egg varies between 40–60 grams and may sometimes reach 70 grams. The shell and shell-membrane together, when carefully cleaned, but still in the moist state, weigh 5–8 grams. The yolk weighs 12–18 and the white 23–34 grams, or about double. The entire egg contains 2.8–7.5, or average 4.6, milligrams of iron oxide, and the quantity of iron can be increased by food rich in iron (HARTUNG [2]).

The white of the egg of cartilaginous and bony fishes contains only traces of true albumin, but consist at least in many fishes, of mucin substance; and the cover of the frog's egg also consists, according to GIACOSA, of mucin. The eggs of the river-perch contain, HAMMARSTEN [3] claims, mucin in the envelope in the unripe state and only mucinogen in the ripe state. The crystalline formations (*yolk-spherules*, or *dotterplättchen*) which have been observed in the egg of the tortoise, frog, ray, shark, and other fishes, and which are described by VALENCIENNES and FRÉMY under the names *emydin*, *ichthin*, *ichthidin*, and *ichthulin*, seem, as above stated in connection with ichthulin, to consist chiefly of phosphoglycoproteins. The *klupeovin* obtained by HUGOUNENQ [4] from the herring's eggs and from which he obtained the three so-called hexone bases and abundant monamino-acids, especially leucine, but not glycocoll or glutamic acid, is to all appearances not a unit body. The eggs of the river-crab and the lobster contain the same pigment as the shell of the animal. This pigment, called *cyanocrystallin*, becomes red on boiling in water.

C. MÖRNER [5] has isolated a substance which he calls *percaglobulin*, from the unripe eggs of the river-perch. It is a globulin and has a strong astringent taste. Especially striking is its property of precipitating certain glycoproteins, such as ovomucoid and ovarial mucoids, and polysaccharides, such as glycogen, gum tragacanth and starch-paste, and of being precipitated by them.

In fossil eggs (of APETNODYTES, PELECANUS, and HALLÆUS) in old guano deposits, a yellowish white, silky, laminated compound has been found which is called *guanovulit*, $(NH_4)_2SO_4+2K_2SO_4+3KHSO_4+4H_2O$, and which is easily soluble in water, but is insoluble in alcohol and ether.

Those eggs which develop outside of the mother-organism must contain all the elements necessary for the young animals. One finds, therefore, in the yolk and white of the egg an abundant quantity of protein bodies of different kinds, and especially phosphorized proteins in the yolk. Further, we also find abundance of phosphatides in the yolk, which seem to occur habitually in all developing cells. The occurrence of glycogen is doubtful, and the carbohydrates are perhaps represented by a very small amount of sugar and glycoproteins. On the contrary, the egg contains a large proportion of fat, which doubtless is important as a source of supply for nourishment and in maintaining respiration for the embryo. The

[1] Arch. f. (Anat. u.) Physiol., 1892.

[2] Zeitschr. f. Biol. **43**.

[3] Giacosa, Zeitschr. f. physiol. Chem., **7**; Hammarsten, Skand. Arch. f. Physiol., **17**.

[4] Valenciennes and Frémy, cited from Hoppe-Seyler, Physiol. Chem., p. 77; Hugounenq, Bull. soc. chim. (3), **33**, and Compt. rend., **143**.

[5] Zeitschr. f. physiol. Chem., **40**

cholesterin or at least the lutein can hardly have a direct influence on the development of the embryo. The egg also seems to contain the mineral bodies necessary for the development of the young animal. The lack of phosphoric acid is compensated by an abundant amount of phosphorized organic substance, and the nucleoalbumin containing iron, from which the hæmatogen (see page 598) is formed, is doubtless, as BUNGE claims, of great importance in the formation of the hæmoglobin containing iron. The silicic acid, necessary for the development of the feathers, is also found in the egg.

During the period of incubation the egg loses weight, chiefly due to loss of water. The quantity of solids, especially the fat and the proteins, diminishes, and the egg gives off carbon dioxide, but TANGL disproves the older claim of LIEBERMANN [1] that nitrogen or a nitrogenous substance is given off. On the contrary a corresponding absorption of oxygen takes place, and it is found that during incubation a respiratory exchange of gases occurs.

As BOHR and HASSELBALCH have shown by exact investigations, the elimination of carbon dioxide is very small in the first days of incubation; on the fourth day the carbon-dioxide production gradually increases, and after the ninth day it augments in the same proportion as the weight of the fœtus. Calculated upon 1 kilogram weight for one hour it is, from the ninth day on, about the same as in the full-grown hen. HASSELBALCH [2] has also shown that the fertilized hen's egg not only gives off nitrogen the first five or six hours of incubation, but also some oxygen, and that we are here dealing with an oxygen production which runs parallel with the cell-division. It is not known whether this oxygen formation connected with the life of the cell is a fermentative or a so-called vital process.

While the quantity of dry substance in the egg during this period always decreases, the quantity of mineral bodies, protein, and fat always increases in the embryo. The increase in the amount of fat in the embryo depends, in great part upon a taking up of the nutritive yolk in the abdominal cavity. The weight of the shell and the quantity of lime-salts contained therein does not remain unchanged, according to the recent investigations of TANGL.[3] The egg-shell (lime shell and shell membrane) of a hen's egg weighing 60 grams loses (calculated on the dry) during incubation about 0.4 gram, of which 0.15 gram is calcium and 0.2 gram is organic substance.

Very complete and careful chemical investigation on the development

[1] Tangl and v. Mituch, Pflüger's Arch., **121**; Liebermann, *ibid.*, **43**.

[2] Bohr and Hasselbalch, Maly's Jahresber., **29**; Hasselbalch, Skand. Arch. f. Physiol., **13**.

[3] Tangl with Hammerschlag, Pflüger's Arch., **121**.

of the embryo of the hen have been made by LIEBERMANN.[1] From his researches we may quote the following: In the earlier stages of the development, tissues very rich in water are formed, but upon the continuation of the development the quantity of water decreases. The absolute quantity of the bodies soluble in water increases with the development, while their relative quantity, as compared with the other solids, continually decreases. The quantity of the bodies soluble in alcohol quickly increases. A specially important increase is noticed in the fat, whose quantity is not very great even on the fourteenth day, but after that it becomes considerable. The quantity of protein bodies and albuminoids soluble in water grows continually and regularly in such a way that their absolute quantity increases, while their relative quantity remains nearly unchanged. LIEBERMANN found no gelatin in the embryo of the hen. The embryo does not contain any gelatin-forming substance until the tenth day, and from the fourteenth day on it contains a body which, when boiled with water, gives a substance similar to chondrin. A body similar to mucin occurs in the embryo when about six days old, but then disappears. The quantity of hæmoglobin shows a continual increase compared with the weight of the body. LIEBERMANN found that the relation of the hæmoglobin to the body weight was 1:728 on the eleventh day and 1:421 on the twenty-first day.

By means of BERTHELOT'S thermometric methods TANGL[2] has determined the chemical energy present at the beginning and end of the development of the embryo of the sparrow's and hen's eggs. The difference was considered as work of development. He found that the chemical energy necessary for the development of each gram of ripe hen's embryo (Plymouth) was equal to 0.805 Cal. This energy originated chiefly from the fat. Of the total chemical energy utilized, about 70 per cent was used for the embryo and about 30 per cent remained in the yolk. Of the utilized energy about two-thirds was used in the construction of the embryo and about one-third transformed into other forms of energy as work of development.

By their investigations on the development of the trout egg TANGL and FARKAS[3] have found that the loss in weight of each egg which had an average weight of 88 milligrams was 4.9 milligrams during the 42 days of incubation, of which 4.11 milligrams was water and 0.722 milligram dry substance with 0.367 milligram C. The eggs lose no nitrogen and no fat. The fat content increases a little, and indeed, as these authors believe, at the expense of the proteins. The chemical energy used during development was 6.68 gram-calories.

The highly interesting investigations made by LOEB upon the fer-

[1] l. c. [2] Pflüger's Arch., 93 and 121. [3] Pflüger's Arch., 104.

tilization, development and artificial parthogenesis of the eggs of sea animals have already been discussed in connection with the physico-chemical processes and action of ions (Chapter II). Here it will be sufficient to call attention to the fact that LOEB believes that a synthesis of the nuclear substance, i.e., of nucleoproteins from the constituents of the yolk, is the means of stimulating development, and that oxidation processes direct the proper path of the nuclear syntheses. The setting free of the nuclear syntheses and these oxidation processes also depend upon a change in the peripheral layers of the egg, which is in general followed by a formation of a membrane, and this latter seems to be brought about by zytolytically acting bodies and the liquefaction of a lipoid. The impulse for the development of the egg is according to this assumption the liquefaction of a lipoid on the surface of the egg, and from this follows another assumption that the head of the spermatozoa contain a lipoid-liquefying substance.

The **placenta** has recently been the subject of several investigations. This tissue contains a protein which coagulates at 60–65° C. (BOTTAZZI and DELFINO) whose relation to the **nucleoprotein,** found by others, is not clear. The protein found by SAVARÉ contained 0.45 per cent phosphorus. The nucleic acid studied by KIKKOJI,[1] which is very similar to the thymus nucleic acid, originates from this nucleoprotein. *Glycogen* occurs regularly in the placenta and MOSCATI believes the human placenta contains 5 p. m. glycogen. After removal the glycogen diminishes, and after 24 hours it has disappeared. According to LOCHHEAD and CRAMER[2] the quantity of glycogen in the placenta is not increased by food rich in carbohydrate. In the fœtus (rabbits) the above authors found that the placenta is a storage organ for glycogen until the second half of the gestation period, when the liver begins to functionate in this direction. From this time on the quantity of glycogen in the placenta diminishes.

Enzymes of various kinds, proteolytic as well as lipolytic (monobutyrase), amylases and oxidases have been found in the placenta (ASCOLI, RAINERI, BERGELL and LIEPMANN, SAVARÉ[3]). In the edges of the placenta of the bitch and of cats an orange-colored, crystalline pigment (bilirubin) and a green, amorphous pigment, whose relation to biliverdin is not clear, have been found.[4]

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[1] Bottazzi and Delfino, Centralbl. f. Physiol., **18**, 114; Savaré, Hofmeister's Beiträge, **11**; Kikkoji, Zetischr. f. physiol. Chem., **53**.

[2] Moscati, Zeitschr. f. physiol. Chem., **53**; Lochhead and Cramer, Proc. Roy. Soc., **80** B. (1908).

[3] Ascoli, Centralbl. f. Physiol., **16**; Raineri, Bioch. Centralbl., **4**, 428; Bergell and Liepmann, Münch. med. Wochenschr., 1905; Savaré, Hofmeister's Beiträge, **9**.

[4] See Etti, Maly's Jahresber., **2**, 287, and Preyer, Die Blutkristalle, Jena, 1871.

From the cotyledons of the placenta in ruminants a white or faintly rose-colored creamy fluid, the *uterine milk*, can be obtained by pressure. It is alkaline in reaction, but quickly becomes acid. Its specific gravity is 1.033–1.040. It contains as form-elements fat-globules, small granules, and epithelium-cells. There have been found 81.2–120.9 p. m. solids, 61.2–105.6 p. m. protein, about 10 p. m. fat, and 3.7–8.2 p. m. ash in the uterine milk.

The fluid occurring in the so-called GRAPE-MOLE (Mola racemosa) has a low specific gravity, 1.009–1.012, and contains 19.4–26.3 p. m. solids with 9–10 p. m. protein bodies and 6–7 p. m. ash.

The **amniotic fluid** in women is thin, whitish, or pale yellow; sometimes it is somewhat yellowish brown and cloudy. White flakes separate. The form-elements are *mucus-corpuscles*, *epithelium-cells*, *fat-drops*, and *lanugo hair*. The odor is stale, the reaction neutral or faintly alkaline. The specific gravity is 1.002–1.028.

The amniotic fluid contains the constituents of ordinary transudates. The amount of solids at birth is hardly 20 p. m. In the earlier stages of pregnancy the fluid contains more solids, especially proteins. Among the protein bodies, WEYL found one substance similar to *vitellin*, and with great probability also *seralbumin*, besides small quantities of *mucin*. Enzymes of various kinds (pepsin, diastase, thrombin, lipase) occur, according to BONDI. *Sugar* is regularly found in the amniotic fluid of cows, but not in human beings. In the ox, pig, and goat GÜRBER and GRÜNBAUM also found levulose. The human amniotic fluid also contains some *urea*, *uric acid*, and *allantoin*. The quantity of these may be increased in hydramnion (PROCHOWNICK, HARNACK), which depends on an increased secretion by the kidneys and skin of the fœtus. Creatine and lactates are doubtful constituents of the amniotic fluid. The quantity of urea in the amniotic fluid, is, according to PROCHOWNICK, 0.16 p. m. In the fluid in hydramnion PROCHOWNICK and HARNACK found respectively 0.34 and 0.48 p. m. urea. The chief mass of the solids consists of salts. The quantity of chlorides (NaCl) is 5.7–6.6 p. m. The molecular concentration of the amniotic fluid is somewhat lower than that of the blood, which is no doubt due to a dilution by the fœtal urine (ZANGEMEISTER and MEISSL [1]).

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[1] Weyl, Arch. f. (Anat. u.) Physiol., 1876; Bondi, Centralbl. f. Gynäkol., 1903; Prochownick, Arch. f. Gynäk., **11**, also Maly's Jahresber., **7**, 155; Harnack, Berlin. klin. Wochenschr., 1888, No. 41; Zangemeister and Meissl, Münch. med. Wochenschr., 1903; Gürber and Grünbaum, *ibid.*, 1904.

CHAPTER XIV.

MILK.

THE chemical constituents of the *mammary glands* have been little studied. The cells are rich in protein and *nucleoproteins*. Among the latter we have one that yields pentose and guanine, on boiling with dilute mineral acids, but no other purine base. This *compound protein*, investigated by ODENIUS, contains as an average the following: 17.28 per cent N, 0.89 per cent S, and 0.277 per cent P. MANDEL has made an analysis of the hydrolytic cleavage products of the nucleoprotein of the mammary glands, carefully prepared according to HAMMARSTEN'S method, and finds the amino-acids in nearly the same quantitative proportions as they occur in casein, as determined by FISCHER and ABDERHALDEN and by HART. Besides this compound proteid we have at least one other, as MANDEL and LEVENE and LOEBISCH [1] have isolated a nucleic acid from the mammary gland, which, like the thymonucleic acids, yielded adenine, guanine, thymine, and cytosine. This nucleic acid also gave the pentose reactions and yielded abundance of levulinic acid. Besides this nucleic acid, MANDEL and LEVENE [2] isolated from the glands a glucothionic acid with 2.65 percent S and 4.38 per cent N. We cannot state what relation these substances bear to that constituent of the gland found by BERT, which on boiling with dilute mineral acids yielded a reducing substance. A similar substance, which acts perhaps as a step toward the formation of lactose, has also been observed by THIERFELDER. It is to be expected that these bodies are steps in the formation of milk-sugar; still we have no point of support for such an assumption, and recent investigations seem to indicate that the milk-sugar is produced in the glands by a transformation of the sugar of the blood. *Fat* seems, at least in the secreting glands, to be a never-failing constituent of the cells, and this fat may be observed in the protoplasm as large or small globules similar to milk-globules. The extractive bodies of the mammary glands have been little investigated, but among them are found considerable amounts of *purine bases*. The mammary glands

[1] Odenius, Maly's Jahresber., **30**; Mandel, Bioch. Zeitschr., **22**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46**; Loebisch, Hofmeister's Beiträge, 8.

[2] Zeitschr. f. physiol. Chem., **45**.

also contain a proteolytic enzyme which, according to HILDEBRANDT,[1] occurs to a much greater extent in the active gland as compared with the inactive one.

As human milk and the milk of animals are essentially of the same constitution, it seems best to speak first of the one most thoroughly investigated, namely, cow's milk, and then of the essential properties of the remaining important kinds of milk.[2]

Cow's Milk.

Cow's milk, like every other kind, forms an emulsion which consists of very finely divided fat suspended in a solution consisting chiefly of protein bodies, milk-sugar, and salts. Milk is non-transparent, white, whitish yellow, or in thin layers somewhat bluish white, of a faint, insipid odor and mild, faintly sweetish taste. The specific gravity is 1.028 to 1.0345 at 15° C. The freezing-point is −0.54–0.59° C., average −0.563° C., and the molecular concentration 0.298.

The reaction of perfectly fresh milk is generally amphoteric toward litmus. The extent of the acid and alkaline part of this amphoteric reaction has been determined by different investigators, especially THÖRNER, SEBELIEN, and COURANT.[3] The results differ with the indicators used, and moreover the milk from different animals, as well as that from the same animal at different times during the lactation period, varies slightly. COURANT determined the alkaline part by N/10 sulphuric acid, using blue lacmoid as indicator, and the acid part by N/10 caustic soda, using phenolphthalein as indicator. He found, as an average for the first and last portions of the milking of twenty cows, that 100 cc. milk had the same alkaline reaction toward blue lacmoid as 41 cc. N/10 caustic soda, and the same acid reation toward phenolphthalein as 19.5 cc. N/10 sulphuric acid. The actual reaction of cow's milk, which follows from the electrometric estimation, is, on the contrary, FOA[4] claims, nearly neutral, like the reaction of animal fluids and tissues in general.

Milk gradually changes when exposed to the air, and its reaction becomes more and more acid. This depends on a gradual transformation of the milk-sugar into lactic acid, caused by micro-organisms.

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[1] Bert, Compt. rend., **98**; Thierfelder, Pflüger's Arch., **34**, and Maly's Jahresber., **13**; Hildebrandt, Hofmeister's Beiträge, **5**.

[2] A very complete reference to the literature on milk may be found in Raudnitz's "Die Bestandteile der Milch," in Ergebnisse der Physiol., **2**, Abt. 1. The literature of the last few years may be found in the references by Raudnitz, Monatsschrift f. Kinderheilkunde.

[3] Thörner, Maly's Jahresber., **22**; Sebelien, *ibid.*; Courant, Pflüger's Arch., **50**.

[4] Compt. rend. soc. biolog. (58), **59**, 51.

Perfectly fresh amphoteric milk does not coagulate on boiling, but forms a pellicle consisting of coagulated casein and lime-salts, which rapidly reforms after being removed. Even after passing a current of carbon dioxide through the fresh milk it does not coagulate on boiling. In proportion as the formation of lactic acid advances this behavior changes, and soon a stage is reached when the milk, which has previously had carbon dioxide passed through it, coagulates on boiling. At a second stage it coagulates alone on heating; then it coagulates by passing carbon dioxide alone without boiling; and lastly, when the formation of lactic acid is sufficient, it coagulates spontaneously at the ordinary temperature, forming a solid mass. It may also happen, especially in the warmth, that the casein-clot contracts and a yellowish or yellowish-green acid liquid (acid whey) separates.

Milk may undergo various fermentations. Lactic-acid fermentation, brought about by HÜPPE's lactic-acid bacillus and also other varieties, takes first place. In the spontaneous souring of milk we generally consider the formation of lactic acid as the most essential product, but a formation of succinic acid may also take place, and in certain bacterial decompositions of milk, succinic acid and no lactic acid is formed. The materials from which these two acids are formed are lactose and lactophosphocarnic acid. Besides the lactic acids, the optically inactive as well as the dextro and levo acids, and succinic acid, volatile fatty acids, such as acetic acid, butyric acid, and others, may be formed in the bacterial decomposition of milk.

Milk sometimes undergoes a peculiar kind of coagulation, being converted into a thick, ropy, slimy mass (thick milk). This conversion depends upon a peculiar change in which the milk-sugar is made to undergo a slimy transformation. This transformation, which requires further investigation, is caused by special micro-organisms.

If the milk is sterilized by heating, and contact with micro-organisms prevented, the formation of lactic acid may be entirely stopped. The production of acid may also be prevented, at least for some time, by many antiseptics, such as salicylic acid, thymol, boric acid, and other bodies.

If freshly drawn amphoteric milk is treated with rennet, it coagulates quickly, especially at the temperature of the body, to a solid mass (curd) from which a yellowish fluid (sweet whey) is gradually pressed out. This coagulation occurs without any change in the reaction of the milk, and therefore it is distinct from the acid coagulation.

In cow's milk we find as form-elements a few colostrum corpuscles (see Colostrum) and a few pale nucleated cells. The number of these form-elements is very small compared with the immense amount of the most essential form-constituents, the milk-globules.

The Milk-globules. These consist of extremely small drops of fat whose number is, according to WOLL,[1] 1.06–5.75 millions in 1 c.mm., and

[1] On the Conditions Influencing the Number and Size of Fat-globules in Cow's Milk, Wisconsin Exp. Station, 6, 1892.

whose diameter is 0.0024–0.0046 mm. and 0.0037 mm. as an average for different kinds of animals. It is unquestionable that the milk-globules contain fat, and we consider it as positive that all the milk-fat exists in them. Another disputed question is whether the milk-globules consist entirely of fat or whether they also contain protein.

The observations of ASCHERSON [1] show that drops of fat, when dropped in an alkaline protein solution, are covered with a fine albuminous coat, a so-called *haptogen-membrane*. As milk on shaking with ether does not give up its fat, or only very slowly in the presence of a great excess of ether, and as this takes place very readily after the addition of acids or alkalies, which dissolve proteins, it was formerly thought that the fat-globules of the milk were enveloped in a protein coat. A true membrane has not been detected; and since, when no means of dissolving the protein is resorted to—for example, when the milk is precipitated by carbon dioxide after the addition of very little acetic acid, or when it is coagulated by rennet—the fat can be very easily extracted by ether, the theory of a special albuminous membrane for the fat-globule has been generally abandoned. The observations of QUINCKE [2] on the behavior of the fat-globules in an emulsion prepared with gum have led, at the present time, to the conclusion that each fat-globule in the milk is surrounded by a stratum of casein solution held by molecular attraction, and this prevents the globules from uniting with each other. Everything that changes the physical condition of the casein in the milk or precipitates it must necessarily help the solution of the fat in ether, and it is in this way that the alkalies, acids, and rennet act.

V. STORCH has shown, in opposition to these views, that the milk-globules are surrounded by a membrane of a special slimy substance. This substance is very insoluble, contains 14.2–14.79 per cent nitrogen, and yields a sugar, or at least a reducing substance, on boiling with hydrochloric acid. It is neither casein nor lactalbumin, but it seems to all appearances to be identical with the so-called "stroma substance" detected by RADENHAUSEN and DANILEWSKY. STORCH was able to show, by staining the fat-globules with certain dyes, that this substance enveloped them like a membrane. Recently VÖLTZ has given further proofs of the view that the fat-globules probably have a membrane, which in his opinion is a very labile formation of variable composition. DROOP-RICHMOND and BONNEMA,[3] on the other hand, present several deductions conflicting with STORCH'S theory. If STORCH'S observation

[1] Arch. f. Anat. u. Physiol., 1840.

[2] Pflüger's Arch., **19**.

[3] V. Storch, see Maly's Jahresber., **27**; Radenhausen and Danilewsky, Forschungen auf dem Gebiete der Viehhaltung (Bremen, 1880), Heft 9; Völtz, Pflüger's Arch., **102**; Droop-Richmond, see Chem. Centralbl., 1904, **2**, 356; Bonnema, *ibid.*, 1243.

that the purified fat-globules contain a special protein substance differing from the dissolved proteins of the milk is correct, then the assumption as to a special body forming a membrane or stroma of the fat-globules becomes very probable. The correctness of STORCH'S view has been substantiated very recently by ABDERHALDEN and VÖLTZ.[1] On the acid hydrolysis of the fat-globules they obtained glycocoll, which is absent in the casein as well as in the lactalbumin, and this shows that the fat-globules at least cannot contain these two proteins alone. They must contain another protein, and it is still a question whether besides this they also contain casein and lactalbumin.

The milk-fat which is obtained under the name of butter consists chiefly of *olein* and *palmitin*. Besides these it contains, as triglycerides, *myristic acid*, *stearic acid*, small amounts of *lauric acid*, *arachidic acid*, and *dioxystearic acid*, besides *butyric acid* and *caproic acid*, traces of *caprylic acid* and *capric acid*. RIEGEL claims that triglycerides of volatile fatty acids do not occur, but rather mixed triglycerides of volatile and non-volatile fatty acids. Milk-fat also contains small quantities of *phosphatides*, (*lecithin*) and *cholesterin* and a yellow *coloring-matter*. The quantity of volatile fatty acids in butter is, according to DUCLAUX, on an average about 70 p. m., of which 37–51 p. m. is butyric acid and 30–33 p. m. is caproic acid. The non-volatile fat consists of $\frac{3}{10}$–$\frac{4}{10}$ olein, and the remainder is chiefly palmitin. The composition of butter is not constant, but varies considerably under different circumstances.[2] According to LEMUS[3] the small fat-globules contain more olein and less volatile acids than the large globules.

The *milk-plasma*, or that fluid in which the fat-globules are suspended, contains several different proteins, the statements as to the number and nature of which are somewhat at variance. The three following, *casein*, *lactalbumin*, and *lactoglobulin*, have been most closely studied and are well characterized. The milk-plasma contains two carbohydrates, of which the one, *lactose*, is of great importance. It also contains extractive bodies, traces of *urea*, *creatine*, *creatinine*, *orotic acid*, *hypoxanthine* (?), *cholesterin*, *citric acid* (SOXHLET and HENKEL[4]), and lastly also *mineral bodies* and *gases*.

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[1] Zeitschr. f. physiol. Chem., **59**.

[2] Riegel, Maly's Jahresber., **34**; Duclaux, Compt. rend., **104**. Various statements as to the composition of milk-fat can be found in Koefoed, Bull. d. l'Acad. Roy. Danoise, 1891, and Wanklyn, Chemical News, **63**; Browne, Chem. Centralbl., 1899, **2**, 883. In regard to the elementary composition of milk-fat see Fleischmann and Warmbold, Zeitschr. f. Biol., **50**.

[3] See Maly's Jahresber., **34**.

[4] Cited from Söldner, Die Salze der Milch, etc., Landwirthsch. Versuchsstation, **35**, Separatabzug, 18.

Casein. This protein substance, which thus far has been detected positively only in milk, belongs to the nucleoalbumins, and differs from the albuminates chiefly by its content of phosphorus and by its behavior with the rennet enzyme. Casein from cow's milk has the following composition: C 53.0, H 7.0, N 15.7, S 0.8, P 0.85, and O 22.65 per cent. Its specific rotation is, according to HOPPE-SEYLER, rather variable; in neutral solution it is $(\alpha)_D = -80°$; its faintly alkaline solution has a stronger rotation, namely, -97.8 to $-111.8°$, in a solution of N/10—N/5 NaOH (LONG [1]). The question whether the casein from different kinds of milk is identical or whether there are several caseins cannot be decided by the elementary analysis. According to TANGL and CSÓKÁS [2] mare's and ass's casein seem to be somewhat richer in nitrogen (16.44 and 16.28 per cent respectively) but poorer in sulphur (0.528 and 0.588 per cent) and carbon (52.36 and 52.27 per cent) than the casein from cud chewers. The ass's casein was richer in phosphorus (1.057 per cent) than the mare's or cow's casein (both with 0.887 per cent).

Casein when dry appears like a fine white powder, which has no measurable solubility in pure water (LAQUEUR and SACKUR). Casein is only very slightly soluble in the ordinary neutral-salt solutions. According to ARTHUS it dissolves rather easily in a 1 per cent solution of sodium fluoride, ammonium or potassium oxalate. ROBERTSON thinks that it is more soluble in potassium cyanide and the alkali salts of certain volatile fatty acids such as butyric acid and valeric acid than in solutions of the ordinary neutral salts. It is at least a tetrabasic acid, whose equivalent weight is 1135 according to LAQUEUR and SACKUR, and 1250 according to ROBERTSON. The statements as to the molecular weight are disputed (LAQUEUR and SACKUR, L. and D. VAN SLYKE [3]). It dissolves readily in water with the aid of alkali or alkaline earths, also calcium carbonate, from which it expels carbon dioxide. If casein is dissolved in lime-water and this solution carefully treated with very dilute phosphoric acid until it is neutral in reaction, the casein appears to remain in solution, but is probably only swollen as in milk, and the liquid contains at the same time a large quantity of calcium phosphate without any precipitate or any suspended particles being visible. The casein solutions containing lime are opalescent and have on warming the appearance of milk deficient in fat (which is also true for the salts of casein with the alkaline earths). Therefore it is not impossible that

[1] Hoppe-Seyler, Handb. d. physiol. u. pathol. chem. Analyse, 7. Aufl., 368; Long, Journ. Amer. Chem. Soc., **27**.

[2] Pflüger's Arch., **121**.

[3] Laqueur and Sackur, Hofmeister's Beiträge, **3**; M. Arthus, Thèses presentées à la faculté des sciences de Paris, 1893; Robertson, Journ. of biol. Chem., **2**; L. and D. van Slyke, Amer. Chem. Journ., **38**.

the white color of the milk is due partly to the casein and calcium phosphate. SÖLDNER has prepared two calcium compounds of casein with 1.55 and 2.36 per cent CaO, and these compounds are designated di- and tricalcium casein by COURANT.

Besides the rather earlier investigations on the salts of casein by SÖLDNER, COURANT, RÖHMANN, LAQUEUR, RAUDNITZ [1] and others we have the recent observations and theoretical discussion of ROBERTSON[2] on the composition, nature and dissociation of the caseinates. We can here only refer to this and the earlier investigations.

Casein solutions do not coagulate on boiling, but solutions of casein-lime are covered, like milk, with a pellicle. They are precipitated by very little acid, but the presence of neutral salts retards the precipitation. A casein solution containing salt or ordinary milk requires, therefore, more acid for precipitation than a salt-free solution of casein of the same concentration. The precipitated casein dissolves very easily again in a small excess of hydrochloric acid, but less easily in an excess of acetic acid. The combination between casein and acid, like other protein and acid compounds, is precipitated by neutral salts. These acid solutions are precipitated by mineral acids in excess.[3] Casein is precipitated from neutral solutions or from milk by common salt, containing calcium or magnesium sulphate, in substance, without changing its properties.[4] Metallic salts, such as alum, zinc sulphate, and copper sulphate, completely precipitate the casein from neutral solutions.

On drying at 100° C., casein, according to LAQUEUR and SACKUR, decomposes and splits into two bodies. One of these, called *caseid*, is insoluble in dilute alkalies, while the other, the *isocasein*, is soluble therein. The isocasein is a stronger acid and has other precipitation limits and a rather lower equivalent weight than the casein.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently large amount of lime-salts. In solutions free from lime-salts the casein does not coagulate with rennet, but it is changed so that the solution (even if the enzyme is destroyed by heating) yields a coagulated mass, having the properties of a curd, if lime-salts are added. The rennet enzyme, rennin, has therefore an action on casein even in the absence of lime-salts. These last

[1] Söldner, Die Salze d. Milch, etc., and Maly's Jahresber., **25**; Courant l. c.; Röhmann, Berl. klin. Wochenschr., 1895; Laqueur, l. c., and Hofmeister's Beiträge, **7**; Raudnitz, Ergebn. d. Physiol., **2**, Abt. 1.

[2] Journ. of physik. Chem., **11** and **12**; Journ. of biol. Chem., **5**.

[3] In regard to the acid combinations of casein and the ability to take up acid, see Laxa, Milchwirkch. Centralbl., 1905; Long, Journ. Amer. Chem. Soc., **29**; L. and D. van Slyke, Amer. Chem. Journ., **38**; Robertson, Journ. of biol. Chem., **4**.

[4] See the works of Hammarsten and Schmidt-Nielsen, Hammarsten's Festschrift, 1906.

are only necessary for the coagulation or the separation of the curd, and the process of coagulation is hence a two-phase process. The first phase is the transformation of the casein by the rennin, the second is the visible coagulation caused by the lime-salts. This fact, which was first proven by HAMMARSTEN, was later confirmed by ARTHUS and PAGÈS and recently closely studied by FULD, SPIRO, and LAQUEUR and others.[1]

The curd formed on the coagulation of milk contains large quantities of calcium phosphate. According to SOXHLET and SÖLDNER, the soluble lime-salts are of essential importance only in coagulation, while the calcium phosphate is without importance. COURANT believes that the calcium-casein on coagulation may carry down with it, if the solution contains dicalcium phosphate, a part of this as tricalcium phosphate, leaving mono-calcium phosphate in the solution. A solution of calcium casein is not coagulated by rennin alone but only when soluble lime-salts are added. Contrary to the generally accepted view that the soluble lime-salts are of importance in the coagulation, VAN DAM[2] claims that it is the quantity of lime combined with the casein which is of importance in the coagulation process. The role of the lime-salts in coagulation is not clear, and this follows from the chemical procedure in rennin coagulation.

If one makes use of a pure solution of casein and as pure rennin as possible, then after coagulation it is always found that the filtrate contains very small amounts of a protein, the *whey protein*, which is probably formed in the coagulation. This behavior, which was first shown by HAMMARSTEN, has been substantiated by many others and recently by FULD, SPIRO and SCHMIDT-NIELSEN. Whey protein is generally considered as a proteose substance, and KÖSTER[3] found 13.2 per cent nitrogen therein. In correspondence with these observations casein coagulation with rennin is considered as a cleavage process, in which the chief mass of the casein, sometimes more than 90 per cent, is split off as *paracasein*,[4] a body closely related to casein, and in the presence of sufficient

[1] See Maly's Jahresber., **2** and **4**; also Hammarsten, Zur Kenntniss des Kaseïns und der Wirkung des Labfermentes, Nova Acta Reg. Soc. Scient. Upsala, 1877, Festschrift; Zeitschr. f. physiol. Chem., **22**; Arthus et Pagès, Arch. de Physiol. (5), **2**, and Mém. soc. biol., **43**; Fuld, Hofmeister's Beiträge, **2**, and Ergebnisse der Physiol., **1**, Abt. 1, where a good review of the literature may be found; Spiro, Hofmeister's Beiträge, **6** and **7**, with Reichel, *ibid.*, **7** and 8; Laqueur, *ibid.*, **7**.

[2] Zeitschr. f. physiol. Chem., **58**.

[3] Hammarsten, l. c.; Fuld, Bioch. Zeitschr., **4**, and Hofmeister's Beiträge, **10**; Spiro, Hofmeister's Beiträge, 8; Schmidt-Nielsen, Hammarsten's Festschrift, 1906; Köster, see Maly's Jahresber., **11**, **14**.

[4] It has been proposed to designate the ordinary casein as caseinogen and the curd as casein. Although such a proposition is theoretically correct, it leads in practice to confusion. On this account the author calls the curd paracasein, according to Schulze and Röse (Landwirthsch. Versuchsstat., **31**). A summary of the literature on

amounts of lime-salts the paracasein-lime precipitates out while the proteose-like substance (whey protein) remains in solution.

The paracasein is very similar to casein, but cannot be recoagulated by rennin. As solution of alkali-paracaseinate is much more readily precipitated by $CaCl_2$ than an alkali-caseinate solution of the same concentration, and the precipitation limits for saturated ammonium-sulphate solution, the upper as well as the lower limit, lie, according to LAQUEUR, lower with paracasein than with casein. The internal friction of paracasein solutions is also, in his opinion, less than that of casein solutions and indeed even to 20 per cent.

By continued action of rennin upon paracasein a further transformation has been found in many cases (PETRY, SLOWTZOFF, v. HERWERDEN [1]). This is explained by the presence of another proteolytic enzyme in the (impure) rennin preparation. This assumption seems to be plausible, and we are here probably dealing only with a secondary process which has nothing whatever to do with the true formation of paracasein. Whey protein is also formed after the very short action of rennin, and the continued cleavage occurs with varying speed. Thus SCHMIDT-NEILSEN found that the quantity of whey protein was even 3 per cent of the casein nitrogen after the action of rennet for 15 minutes and only 4.25 per cent after 6 hours action. These and other recent investigations all favor the assumption that the casein coagulation by rennet is a hydrolytic cleavage, but the conditions are not so clear that this can be considered as proven.[2]

Fresh, unchanged milk does not, as is known, coagulate on boiling; but in not too rapid action of rennin a state may be observed in which the milk coagulates on heating (metacasein reaction). A solution of paracasein lactate, according to LAXA, coagulates with rennin the same as a solution of casein lactate, which indicates, he believes,[3] that the paracasein is transformed into casein again by the lactic acid. But as a precipitation of the paracasein from the acid solution is perhaps a pepsin action, the transformation of the paracasein into casein by the lactic acid must not be considered as proven.

In the digestion of casein with pepsin-hydrochloric acid primarily a phosphorized proteose is formed, from which then the pseudonuclein is split off (SALKOWSKI). The quantity thus split off is variable, as shown by the researches of SALKOWSKI, HAHN, MORACZEWSKI, SEBELIEN, and ZAITSCHEK.[4] The amount of phosphorus in the pseudonucleins

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the casein coagulation may be found in E. Fuld, Ergebnisse der Physiol., **1**; Raudnitz, *ibid.*, **2**; and Laqueur, Biochem. Centralbl., **4**, 344.

[1] Petry, Hofmeister's Beiträge, 8; Slowtzoff, *ibid.*, **9**; v. Herwerden, Zeitschr. f. physiol. Chem., **52**.

[2] See also Werncken, Zeitschr. f. Biol., **52**.

[3] Laxa, l. c.

[4] Salkowski, Zeitschr. f. physiol. Chem., **27**; Salkowski and Hahn, Pflüger's Arch.,

obtained also varies considerably. Salkowski considers that the quantity of pseudonuclein split off is dependent upon the relation between the casein and the digestion fluid, e.g., the quantity of the pseudonucleins diminishes as the pepsin-hydrochloric acid increases. In the presence of 500 grams of pepsin-hydrochloric acid to 1 gram of casein Salkowski digested the latter completely without obtaining any pseudonuclein.

In peptic as well as tryptic digestion a part of the organic phosphorus is split off as orthophosphoric acid, the quantity increasing as the digestion progresses. Another part of the phosphorus is retained in organic combination in the proteoses as well as in the true peptones (Salkowski, Biffi, Alexander, Aders Plimmer and Bayliss [1]).

From the products of peptic digestion of casein, after the separation of the pseudonuclein, Salkowski [2] has isolated an acid rich in phosphorus. He considers this a *paranucleic acid.* It is soluble in water, insoluble in alcohol, levorotatory, and contained N 13.25–13.55, and P 4.05–4.31 per cent. The acid differs from the nucleic acids in that it gives the biuret test and a faint xanthoproteic reaction. Presupposing its purity, it is not an acid comparable with the nucleic acids. A still richer product in phosphorus, with 6.9 per cent P, has been isolated as a uranium compound by Reh [3] from the peptic digestive products of casein. He calls this body *polypeptid phosphoric acid.* On hydrolysis this product gave 18.7 per cent nitrogen as diamino-acids, 56.7 per cent as monamino-acids and the remarkably high result 23.8 per cent amido-nitrogen. This pseudonucleic acid also gave the biuret and the xanthoproteic tests and Millon's reaction, and behaved like a protein rich in phosphorus.

Casein may be prepared in the following way: The milk is diluted with 4 vols. of water and the mixture treated with acetic acid to 0.75—1 p. m. Casein thus obtained is purified by repeatedly dissolving in water with the aid of the smallest quantity of alkali possible, by filtering and reprecipitating with acetic acid and thoroughly washing with water. Most of the milk-fat is retained by the filter on the first filtration, and the casein contaminated with traces of fat is purified by treating with alcohol and ether.

Lactoglobulin was obtained by Sebelien from cow's milk by saturating it with NaCl in substance (which precipitated the casein) and saturating the filtrate with magnesium sulphate. As far as it has been investigated

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59; Salkowski, *ibid.*, 63; v. Moraczewski, Zeitschr. f. physiol. Chem., 20; Sebelien *ibid.*, 20; Zaitschek, Pflüger's Arch., 104.

[1] Salkowski, l. c.; Biffi, Virchow's Arch., 152; Alexander, Zeitschr. f. physiol. Chem., 25; Plimmer and Bayliss, Journ. of Physiol., 33.

[2] Zeitschr. f. physiol. Chem., 32.

[3] Hofmeister's Beiträge, 11.

it had the properties of serglobulin; the globulin isolated by TIEMANN [1] from colostrum had nevertheless a markedly low content of carbon, namely, 49.83 per cent.

Lactalbumin was first prepared in a pure state from milk by SEBELIEN. He gives its composition as, C 52.19, H 7.18, N 15.77, S 1.73, O 23.13 per cent. Lactalbumin has the properties of the albumins, and WICHMANN [2] found that it crystallizes in forms similar to ser- or ovalbumin. It coagulates, depending on the concentration and the amount of salt in solution, at 72–84° C. It is similar to seralbumin, but differs from it in having a considerably lower specific rotatory power: $(\alpha)_D = -37°$.

The principle of the preparation of lactalbumin is the same as for the preparation of seralbumin from serum. The casein and the globulin are removed by $MgSO_4$ in substance and the filtrate treated as previously stated (page 255).

The occurrence of other proteins, such as *proteoses* and *peptones*, in milk has not been positively proven. These bodies are easily produced as laboratory products from the other proteins of the milk. Such a laboratory product is MILLON'S and COMAILLE'S *lactoprotein*, which is a mixture of a little casein with changed albumin, and proteose [3] which is formed by chemical action. In regard to *opalisin*, see Human Milk, p. 628.

Milk also contains, SIEGFRIED [4] claims, a *nucleon* related to phosphocarnic acid, which yields fermentation lactic acid (instead of paralactic acid) and a special carnic acid, *orylic acid* (instead of muscle carnic acid), as cleavage products. Lactophosphocarnic acid may be precipitated as an iron compound from the milk freed from casein and coagulable proteins as well as from earthy phosphates.

Milk also contains *enzymes* of various kinds. Of these we must mention *catalases*, *oxidases*, *peroxidases*, and *reductases*, but the statements as to their occurrence in the milk from different animals as well as the question how much of their action is due to micro-organisms are conflicting. An *amylolytic enzyme* which converts starch into maltose occurs, especially, in human milk, while it is absent in cow's milk or occurs only to a slight extent. A *fermentation enzyme* which in the absence of micro-organisms decomposes the lactose into lactic acid, alcohol, and CO_2, occurs, according to STOKLASA [5] and his co-workers, in cow's milk as well as in human milk. Human milk, as well as cow's milk, contains a *lipase* which has the property at least of acting upon monobutyrin. BABCOCK and RUSSEL have

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[1] Zeitschr. f. physiol. Chem., **25**.

[2] Sebelien, Zeitschr. f. physiol. Chem., **9**; Wichmann, *ibid.*, **27**.

[3] See Hammarsten, Maly's Jahresber., **6**, 13.

[4] Zeitschr. f. physiol. Chem., **21** and **22**.

[5] See Chem. Centralbl., 1905, **1**, 107.

found in these two kinds of milk, as well as certain others, a proteolytic enzyme which they call *galactase*, which is allied to trypsin, but differs therefrom in that it develops ammonia from milk even in the early stages of digestion. The occurrence of such an enzyme is denied by ZAITSCHEK and v. SZONTAGH, but on the other hand VANDEVELDE, DE WAELE, and SUGG [1] confirm the occurrence of a proteolytic enzyme in milk.

Orotic acid, $C_5H_{11}N_2O_4.2H_2O$, is the name given by BISCARO and BELLONI [2] to a new constituent of milk which they have discovered. This acid, which can be precipitated by basic lead acetate from whey free from protein, is slightly soluble in water, crystalline, and gives several crystalline salts. The monomethyl and ethyl esters of this acid are also known. It yields urea on treatment with potassium permanganate.

Lactose, MILK-SUGAR, $C_{12}H_{22}O_{11}+H_2O$. This sugar, on hydrolysis, can be split into two hexoses, *dextrose* and *galactose*. It yields mucic acid, besides other organic acids, by the action of dilute nitric acid. Levulinic acid is formed, besides formic acid and humin substances, by the stronger action of acids. By the action of alkalies, among other products we find lactic acid and pyrocatechin.

Milk-sugar occurs, as a rule, only in milk, but it has also been found in the urine of pregnant women on stagnation of milk, as well as in the urine after partaking of large quantities of the same sugar.

Lactose, of which, according to TANRET,[3] there are three modifications, occurs ordinarily as colorless rhombic crystals with 1 molecule of water of crystallization, which is driven off by slowly heating to 100° C., but more easily at 130–140° C. On quickly boiling down a milk-sugar solution, anhydrous milk-sugar separates out. Milk-sugar dissolves in 6 parts cold or in 2.5 parts boiling water; it has a faintly sweetish taste. It does not dissolve in ether or absolute alcohol. Its solutions are dextrogyrate. The rotatory power, which on heating the solution to 100° C. becomes constant, is $(\alpha)_D = +52.5°$. Milk-sugar combines with bases; the alkali combinations are insoluble in alcohol.

Milk-sugar is not fermentable with pure yeast. It undergoes, on the contrary, alcoholic fermentation by the action of certain schizomycetes, and E. FISCHER [4] found that the milk-sugar is first split into dextrose and galactose by an enzyme, *lactase*, existing in the yeast. The preparation of milk-wine, "*kumyss*," from mare's milk and "*kephir*" from cow's milk is based upon this fact. Other micro-organisms also take part in this change, causing a lactic-acid fermentation of the milk-sugar.

[1] Babcock and Russel, Centralbl. f. Bakt. u. Parisitenkunde (II), **6**, and Maly's Jahresber., **31**; Zaitschek and v. Szontagh, Pflüger's Arch., **104**; Vandevelde, de Waele, and Sugg, Hofmeister's Beiträge, **5**.

[2] See Chem. Centralbl., 1905, **2**, 63.

[3] Bull. soc. chim. (3), **13**. See also Hudson, Journ. Amer. Chem. Soc., **30**.

[4] Ber. d. d. Chem. Gesellsch., **27**.

Lactose responds to the reactions of dextrose, such as MOORE'S,[1] TROMMER'S and RUBNER'S, and the bismuth test. It also reduces mercuric oxide in alkaline solutions. After warming with phenylhydrazine acetate it gives on cooling a yellow crystalline precipitate of phenyl lactosazone, $C_{24}H_{32}N_4O_9$. It differs from cane-sugar by giving positive reactions with MOORE'S or TROMMER'S and the bismuth test, and also in that it does not darken when heated to 100° C. with anhydrous oxalic acid. It differs from dextrose and maltose by its solubility and crystalline form, but especially by its not fermenting with yeast and by yielding mucic acid with nitric acid.

The osazone obtained with phenylhydrazine acetate, which melts at 200° C., differs from the other osazones by being inactive when 0.2 gram is dissolved in 4 cc. of pyridine and 6 cc. of absolute alcohol and viewed through a layer 10 centimetres long (NEUBERG [2]).

For the preparation of milk-sugar we make use of the by-product in the preparation of cheese, the sweet whey. The protein is removed by coagulation with heat, and the filtrate evaporated to a syrup. The crystals which separate after a certain time are recrystallized from water after decolorizing with animal charcoal. A pure preparation may be obtained from the commercial milk-sugar by repeated recrystallization. The quantitative estimation of milk-sugar may be performed either by the polaristrobometer or by means of titration with FEHLING'S solution. Ten cc. of FEHLING'S solution are reduced by 0.0676 gram of milk-sugar in 0.5–1.5 per cent solution after boiling for six minutes. (In regard to FEHLING'S solution and the titration of sugar see larger hand-books.)

From the non-correspondence between the quantity of sugar in the milk as determined by polarization and gravimetrically, when the polarization results are always higher, SEBELIEN [3] has concluded that the milk must contain a second reducing substance which polarizes stronger than lactose. This substance is probably a pentose and occurs to a very slight extent in ordinary milk, 0.25–0.35 p. m. (SEBLIEN and SUNDE), and more in colostrum, 0.5 p. m.

RITTHAUSEN found another carbohydrate in milk which is soluble in water, non-crystallizable, which has a faint reducing action, and which yields on boiling with an acid a body having a greater reducing power. LANDWEHR considers this as animal gum, and BÉCHAMP [4] as dextrin.

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[1] The well-known beautiful red color, which milk produces after the addition of alkali, at the room temperature and to which recently attention has been called by Gautier, Morel, and Monod (Compt. rend. soc. biol., **60** and **62**), and Krüger (Zeitschr. f. physiol. Chem., **50**) is a Moore's reaction modified by the presence of protein and perhaps also other milk constituents.

[2] Ber. d. d. Chem. Gesellsch., **32**.

[3] Sebelien, Hammarsten's Festschrift, 1906; with Sunde, Zeitschr. f. angew. Chem., **21**.

[4] Ritthausen, Journ. f. prakt. Chem. (N. F.), **15**; Landwehr, Zeitschr. f. physiol. Chem. 8, **9**; Béchamp, Bull. Soc. chim. (3), **6**.

The *mineral bodies* of milk will be treated in connection with its quantitative composition.

The methods for the quantitative analysis of milk are very numerous, and as they cannot all be treated here, we will give the chief points of a few of the methods considered most trustworthy and most frequently employed.

In determining the *solids* a carefully weighed quantity of milk is mixed with an equal weight of heated quartz sand, fine glass powder, or asbestus. The evaporation is first done on the water-bath and finished in a current of carbon dioxide or hydrogen not above 100° C.

The *mineral bodies* are determined by incinerating the milk, using the precautions mentioned in the text-books. The results obtained for the phosphoric acid are incorrect on account of the burning of phosphorized bodies, such as casein and lecithin. We must, therefore, according to SÖLDNER, subtract in round numbers 25 per cent from the total phosphoric acid found in the milk. The quantity of sulphate in the ash also depends on the combustion of the proteins.

In the determination of the *total amount of proteins* RITTHAUSEN'S method is employed, namely, the precipitation of the milk with copper sulphate according to the modification suggested by MUNK.[1] He precipitates all the proteins by means of cupric hydoxide at boiling heat, and determines the nitrogen in the precipitate by means of KJELDAHL'S method. This modification gives more exact results.

According to SEBELIEN'S method, three to four grams of milk are diluted with an equal volume of water, a little common-salt solution added, and the proteins precipitated with an excess of tannic acid. The precipitate is washed with cold water, and then the quantity of nitrogen determined by KJELDAHL'S method. The total nitrogen found when multiplied by 6.37 (casein and lactalbumin contain both 15.7 per cent nitrogen) gives the total quantity of proteins. This method, which is readily performed, gives very good results. I. MUNK used this method in the analysis of woman's milk. In this case the quantity of nitrogen found must be multiplied by 6.34. G. SIMON[2] found that the precipitation with tannic acid, also with phosphotungstic acid, is the simplest and most accurate. The objection to this and other methods in which the proteins are precipitated is that perhaps other bodies (extractives) may be carried down at the same time (CAMERER and SÖLDNER[3]). It is not known to what extent this takes place.

A part of the nitrogen in the milk exists as extractives, and this nitrogen is calculated as the difference between the total nitrogen and the protein nitrogen. According to MUNK'S analyses about $\frac{1}{16}$ of the total nitrogen belongs to the extractives in cow's milk, and $\frac{1}{11}$ in woman's milk. CAMERER and SÖLDNER determine the nitrogen in the filtrate from the tannic-acid precipitate by KJELDAHL'S method, and also according to HÜFNER'S method (hypobromite). In this way they found 18 milligrams of nitrogen according to HÜFNER (urea, etc.) in 100 grams of cow's milk.

To determine the *casein* and *albumin* separately we may make use of the method first suggested by HOPPE-SEYLER and TOLMATSCHEFF,[4]

[1] Ritthausen, Journ. f. prakt. Chem. (N. F.), **15**; I. Munk, Virchow's Arch., **134**.

[2] Sebelien, Zeitschr. f. physiol. Chem., **13**; Simon, *ibid.*, **33**.

[3] Zeitschr. f. Biologie, **33** and **36**.

[4] Hoppe-Seyler, Med. chem. Untersuch., 272.

in which the casein is precipitated by magnesium sulphate. According to SEBELIEN the milk is diluted with its own volume of a saturated magnesium-sulphate solution, then saturated with the salt in substance, and the precipitate then filtered and washed with a saturated magnesium-sulphate solution. The nitrogen is determined in the precipitate by KJELDAHL'S method, and the quantity of casein (+globulin) determined by multiplying the result by 6.37. The quantity of lactalbumin may be calculated as the difference between the casein and the total proteins found. The lactalbumin may also be precipitated by tannic acid from the filtrate from the casein precipitate containing $MgSO_4$, after diluting with water, the nitrogen determined by KJELDAHL'S method and the result multiplied by 6.37.

SCHLOSSMANN [1] suggests an alum solution, which precipitates the casein, in order to separate the casein from the other proteins, and the albumin is then precipitated from the filtrate by tannic acid. The nitrogen in the precipitate is determined by the KJELDAHL method. This method has recently been tested by SIMON and he recommends it highly.

The *fat* is gravimetrically determined by thoroughly extracting the dried milk with ether, evaporating the ether from the extract, and weighing the residue. The fat may be determined by aerometric means by adding alkali to the milk, shaking with ether, and determining the specific gravity of the fat solution by means of SOXHLET'S apparatus. In determining the amount of fat in a large number of samples the lactocrit of DE LAVAL may be used with success. There are numerous other methods for estimating milk-fat, but they cannot be considered here.

In determining the *milk-sugar* the proteins are first removed. For this purpose we precipitate either with alcohol, which must be evaporated from the filtrate, or by diluting with water, and removing the casein by the addition of a little acid, and the lactalbumin by coagulation at boiling heat. The sugar is determined by titration with FEHLING'S or KNAPP'S solution (see Chapter XV). The principle of the titration is the same as for the titration of sugar in the urine; 10 cc. of FEHLING'S solution correspond to 0.0676 gram of milk-sugar; 10 cc. of KNAPP'S solution correspond to 0.0311–0.0310 gram of milk-sugar, when the saccharine liquid contains about ½–1 per cent of sugar. In regard to the *modus operandi* of the titration we must refer the reader to more complete works.

Instead of these volumetric determinations other methods of estimation, such as ALLIHN'S method, the polariscope method, and others, may be used. In calculating the analysis or in determining the solids it is of importance to remember, as suggested by CAMERER and SÖLDNER, that the milk-sugar in the residue is anhydrous. Many other methods for determining the milk-sugar have been suggested and recommended.

The *quantitative composition* of cow's milk is naturally very variable. The average obtained by KÖNIG [2] is as follows in 1000 parts:

Water.	Solids.	Casein.	Albumin.	Fats.	Sugar.	Salts.
871.7	128.3	30.2	5.3	36.9	48.8	7.1
		35.5				

[1] Zeitschr. f. physiol. Chem., 22.

[2] Chemie der menschlichen Nahrungs- und Genussmittel, 4. Aufl.

The quantity of *mineral bodies* in 1000 parts of cow's milk is, according to the analyses of SÖLDNER, as follows: K_2O 1.72, Na_2O 0.51, CaO 1.98, MgO 0.20, P_2O_5 1.82 (after correction for the pseudonuclein), Cl 0.98 grams. BUNGE [1] found 0.0035 gram Fe_2O_3. According to SÖLDNER the K, Na, and Cl are found in the same quantities in whole milk as in milk-serum. Of the total phosphoric acid 36–56 per cent and of the lime 53–72 per cent is not in simple solution. A part of this lime is combined with the casein; the remainder is found united with the phosphoric acid as a mixture of dicalcium and tricalcium phosphates which is kept dissolved or suspended by the casein. The bases are in excess of the mineral acids in the milk-serum. The excess of the first is combined with organic acids, which correspond to 2.5 p. m. citric acid (SÖLDNER).

The *gases* of the milk consist chiefly of CO_2, besides a little N and traces of O. PFLÜGER [2] found 10 vols. per cent CO_2 and 0.6 vol. per cent N calculated at 0° C. and 760 mm. pressure.

The variation in the composition of cow's milk depends on several circumstances.

The colostrum, or the milk which is secreted before calving and in the first few days after, is yellowish, sometimes alkaline, but often acid, of higher specific gravity, 1.046–1.080, and richer in solids than ordinary milk. The colostrum contains, besides fat-globules, an abundance of colostrum-corpuscles—nucleated granular cells 0.005–0.025 mm. in diameter with abundant fat-granules and fat-globules. The fat of colostrum has a somewhat higher melting-point and is poorer in volatile fatty acids than the fat from ordinary milk (NILSON [3]). The iodine equivalent of the colostrum-fat is higher than that of milk-fat. The quantity of cholesterin and lecithin is generally greater. The most apparent difference between it and ordinary milk is that colostrum coagulates on heating to boiling because of the absolutely and relatively greater quantities of globulin and albumin that it contains.[4] The composition of colostrum varies considerably. KÖNIG gives as average the following figures in 1000 parts:

Water.	Solids.	Casein.	Albumin and Globulin.	Fat.	Sugar.	Salts.
746.7	253.3	40.4	136.0	35.9	26.7	15.6

The influence which food exercises upon the composition of milk will be discussed in connection with the chemistry of the milk secretion.

In the following table is given the average composition of skimmed milk and certain other preparations of milk:

	Water.	Proteins.	Fat.	Sugar.	Lactic Acid.	Salts.
Skimmed milk.....	906.6	31.1	7.4	47.5	...	7.4
Cream.....	655.1	36.1	267.5	35.2	...	6.1
Buttermilk..	902.7	40.6	9.3	37.3	3.4	6.7
Whey.....	932.4	8.5	2.3	47.0	3.3	6.5

[1] Zeitschr. f. Biologie, **10**. [2] Pflüger's Arch., **2**. [3] See Maly's Jahresber., **21**.

[4] See Sebelien, Maly's Jahresber., **18**, and Tiemann, Zeitschr. f. physiol. Chem., **25**. See also Simon, *ibid.*, **33**; Winterstein and Strickler, *ibid.*, **47**.

KUMYSS and KEPHIR are obtained, as above stated, by the alcoholic and lactic-acid fermentation of the milk-sugar, the former from mare's milk and the latter from cow's milk. Large quantities of carbon dioxide are formed thereby, and besides this the protein bodies of the milk are partly converted into proteoses and peptones, which increase the digestibility. The quantity of lactic acid in these preparations may be about 10–20 p. m. The quantity of alcohol varies from 10 to 35 p. m.

Milk of Other Animals. GOAT'S milk has a more yellowish color and another, more specific, odor than cow's milk. The coagulum obtained by acid or rennet is more solid and is harder than that from cow's milk. SHEEP'S milk is similar to goat's milk, but has a higher specific gravity and contains a greater amount of solids.

MARE'S milk is alkaline and contains a casein which is not precipitated by acids in lumps or solid masses, but, like the casein from woman's milk, in fine flakes. This casein is only incompletely precipitated by rennet, and it is very similar also in other respects to the casein of human milk. In BEIL'S [1] opinion the casein from mare's and cow's milk is the same, and the different behavior of the two varieties of milk is due to varying amounts of salts and to a different relation between the casein and the albumin. This does not agree with the investigations of ZAITSCHEK and v. SZONTAGH, who find that the casein from mare's milk, like that from human and ass's milk, is digested by pepsin-hydrochloric acid without leaving a residue. The milk of the ASS is claimed by earlier authorities to be similar to human milk, but SCHLOSSMANN finds it considerably poorer in fat. The researches of ELLENBERGER give similar results, and show great similarity between ass's milk and human milk. The average results were 15 p. m. protein with 5.3 p. m. albumin and 9.4 p. m. casein. This latter, like human casein, does not yield any pseudonuclein on pepsin digestion, which agrees well with the above-mentioned investigations of ZAITSCHEK. The quantity of nucleon was about the same as in woman's milk. The quantity of fat was 15 p. m., and the sugar was 50–60 p. m. REINDEER milk is characterized, according to WERENSKIOLD,[2] by being very rich in fat, 144.6–197.3 p. m., and casein, 80.6–86.9 p. m.

The milk of CARNIVORA (the bitch and cat) is acid in reaction and very rich in solids. The composition of the milk of these animals varies with the composition of the food.

To illustrate the composition of the milk of other animals the following figures, the compilation of KÖNIG, are given. As the milk of each kind of animal may have a variable composition, these figures should only be considered as examples of the composition of milk of various kinds:[3]

Milk of the	Water.	Solids.	Proteins.	Fat.	Sugar.	Salts.
Dog	754.4	245.6	99.1	95.7	31.9	7.3
Cat	816.3	183.7	90.8	33.3	49.1	5.8
Goat	869.1	130.9	36.9	40.9	44.5	8.6
Sheep	835.0	165.0	57.4	61.4	39.6	6.6
Cow	871.7	128.3	35.5	36.9	48.8	7.1
Horse	900.6	99.4	18.9	10.9	66.5	3.1
Ass	900.0	100.0	21.0	13.0	63.0	3.0
Pig	823.7	167.3	60.9	64.4	40.4	10.6
Elephant	678.5	321.5	30.9	195.7	88.5	6.5
Dolphin	486.7	513.3	....	437.6	....	4.6
Whale[4]	698.0	302.0	94.3	194.0	traces	9.9

[1] Studien über die Eiweissstoffe des Kumys und Kefirs, St. Petersburg, 1886 (Ricker).

[2] Zaitschek, l. c.; Schlossmann, Zeitschr. f. physiol. Chem., **22**; Ellenberger, Arch. f. (Anat. u.) Physiol., 1899 and 1902; Werenskiold, Maly's Jahresber., **25**.

[3] Details in regard to the milk of different animals may be found in Pröscher, Zeitschr. f. physiol. Chem., **24**; Abderhalden, *ibid.*, **27**. In regard to pig milk, see Zuntz and Ostertag, Landw. Jahresb., **37**.

[4] Scheibe, cited in Bioch. Centralbl., **7**, 553.

Human Milk.

Woman's milk is amphoteric in reaction. According to COURANT its reaction is relatively more alkaline than cow's milk, but it has nevertheless a lower absolute reaction for alkalinity as well as for acidity. He found between the tenth day and the fourteenth month after confinement practically constant results. The alkalinity, as well as the acidity, was a little lower than in childbed. One hundred cc. of the milk had the same average alkalinity as 10.8 cc. N/10 caustic soda, and the same acidity as 3.6 cc. N/10 acid. The relation between the alkalinity and the acidity in woman's milk was as 3:1, and in cow's milk as 2.1:1. The actual reaction determined electrometrically is, according to FOÀ,[1] still nearly neutral, like the other kinds of milk.

Human milk also contains fewer fat-globules than cow's milk, but they are larger in size. The specific gravity of woman's milk varies between 1.026 and 1.036, generally between 1.028 and 1.034. It is highest in well-fed and lowest in poorly fed women. The freezing-point is lowered on an average 0.589° C., according to WINTER and PARMENTIER [2] constant at 0.55°, and the molecular concentration is 0.318.

The fat of woman's milk has been investigated by RUPPEL. It forms a yellowish-white mass, similar to ordinary butter, having a specific gravity of 0.966 at 15°. It melts at 34.0° C. and solidifies at 20.2° C. The following fatty acids can be obtained from the fat, namely, butyric, caproic, capric, myristic, palmitic, stearic, and oleic acids. The fat from woman's milk is, according to RUPPEL and LAVES,[3] relatively poor in volatile fatty acids. The non-volatile fatty acids consist of one-half oleic acid, while among the solid fatty acids myristic and palmitic acids are found to a greater extent than stearic acid.

The essential qualitative difference between woman's and cow's milk seems to lie in the proteins or in the more accurately determined *casein.* A number of both the earlier and more recent investigators [4] claim that the casein from woman's milk has other properties than that from cow's milk. The essential differences are the following: The casein from woman's milk is precipitated with greater difficulty with acids or salts. It does not coagulate uniformly in the milk after the addition of rennet, which depends, essentially, upon the low amount of lime salts and casein contained in the milk.[5] It may be precipitated by gastric juice, but

[1] Compt. rend. soc. biolog., **58**.

[2] See Maly's Jahresber., **34**.

[3] Ruppel, Zeitschr. f. Biologie, **31**; Laves, Zeitschr. f. physiol. Chem., **19**.

[4] See Biedert, Untersuchungen über die chemischen Unterschiede der Menschen- und Kuhmilch (Stuttgart), 1884; Langgaard, Virchow's Arch., **65**; Makris, Studien über die Eiweisskörper der Frauen- und Kuhmilch, Inaug.-Diss. Strassburg, 1876.

[5] See among others Bienenfeld, Bioch. Zeitschr., **7**, and Fuld and Wohlgemuth, *ibid.*, 8.

dissolves completely and easily in an excess of juice; the casein precipitate produced by an acid is more easily soluble in an excess of the acid; and lastly, the clot formed from the casein of woman's milk does not appear in such large and coarse masses as in the casein from cow's milk, but is more loose and flocculent. This last-mentioned fact is of great importance, since it explains the generally admitted fact of the easy digestibility of the casein from woman's milk. We are not clear as to this difference between the digestibility of the cow's casein and human casein, as the first seems to be utilized in the intestinal tract of the infant to the same extent as human casein (P. Müller, Rubner and Heubner [1]).

The question as to whether the above-mentioned variations depend on a decided difference in the two caseins or only on an unequal relation between the casein and the salts in the two kinds of milk, or upon other circumstances, has not been decided as yet. According to Szontagh and Zaitschek and also Wróblewsky, the casein from human milk does not yield any pseudonuclein on peptic digestion, and hence it cannot be a nucleoalbumin. Wróblewsky found the following for the composition of casein from woman's milk: C 52.24, H 7.32, N 14.97, P 0.68, S 1.117 per cent. Langstein and Bergell obtained much lower figures for N, S and especially P, namely, 14.34, 0.85 and 0.27 per cent respectively. According to Kobrak [2] woman's casein yields some pseudonuclein, and with repeated solution in alkali and precipitation by an acid it becomes more and more like cow's casein. He therefore suggests the possibility that woman's casein is a compound between a nucleoalbumin and a basic protein.

Woman's milk also contains lactalbumin, besides the casein, and a protein substance, very rich in sulphur (4.7 per cent) and relatively poor in carbon, which Wróblewsky calls *opalisin*. The statements as to the occurrence of proteoses and peptones are conflicting as in many other cases. No positive proof as to the occurrence of proteoses and peptones in fresh milk has been given.

Because of the properties and low amount of casein in human milk it is often difficult to precipitate it with acid and to prepare it, but this can easily be accomplished by dialysis. A number of methods have been suggested for the preparation of human casein. Fuld and Wohlgemuth recommend the freezing of the milk previous to precipitation, so that the casein masses become larger to a certain extent and the precipitation becomes easier. Engel [3] recommends dilution with

[1] Müller, Zeitschr. f. Biologie, **39**; Rubner and Heubner, *ibid.*, **37**.

[2] Szontagh, Maly's Jahresber., 22; Zaitschek, l. c.; Wróblewsky, Beiträge zur Kenntniss des Frauenkaseins, Inaug.-Diss. Bern, 1894, and Ein neuer eiweissartiger Bestandteil der Milch, Anzeiger der Akad. d. Wiss. in Krakau, 1898; Kobrak, Pflüger's Arch., 80; Langstein and Bergell, cited in Bioch. Centrabl., 8, 323.

[3] Fuld and Wohlgemuth, Bioch. Zeitschr., 5; Engel, *ibid.*, 14.

water to 5 volumes and the addition of 60–80 cc. N/10 acetic acid for each 100 cc. milk. The mixture is first cooled for 2–3 hours and then, after shaking, warmed on the water-bath to 40° for a few minutes.

Even after those differences are eliminated which depend on the imperfect analytical methods employed, the *quantitative composition of woman's milk* is variable to such an extent that it is impossible to give any average results. The numerous analyses, especially those made on a large number of samples by Pfeiffer, Adriance, Camerer and Söldner,[1] have positively shown that woman's milk is essentially poorer in proteins but richer in sugar than cow's milk. The quantity of protein varies between 10–20 p. m., often amounting to only 15–17 p. m. or less, and is dependent upon the length of lactation (see below). The quantity of fat also varies considerably, but ordinarily amounts to 30–40 p. m. The quantity of sugar should not be below 50 p. m., but may rise to even 80 p. m. About 60 p. m. may be considered as an average, but it should be borne in mind that the quantity of sugar is also dependent upon the length of lactation, as it increases with duration. The amount of mineral bodies varies between 2 and 4 p. m.

From a quantitative standpoint, the most essential differences between woman's and cow's milk are the following: As compared with the quantity of albumin, the quantity of casein is not only absolutely but also relatively smaller in woman's milk than in cow's milk, while the latter is poorer in milk-sugar. Human milk is richer in lecithin, at least relatively to the amount of protein. Burow found 0.49–0.58 p. m. lecithin in cow's milk and 0.58 p. m. in woman's milk, which corresponds to 1.40 per cent for the first milk and 3.05 per cent for the second, calculated on the percentage of protein. Nerking and Haensel found as average for lecithin in cow's milk 0.63 p. m. and in woman's milk 0.50 p. m. Koch found that both human milk and cow's milk contain lecithin as well as cephalin. The total quantity of both bodies in human milk was 0.78 p. m. and in cow's milk 0.72–0.86 p. m. The quantity of nucleon is greater in woman's milk. Wittmaack claims that cow's milk contains 0.566 p. m. nucleon, and woman's milk 1.24 p. m., and according to Valenti the quantity of nucleon in human milk is indeed still higher. Siegfried finds that the nucleon phosphorus amounts to 6.0 per cent of the total phosphorus in cow's milk and 41.5 per cent in woman's milk, and also that in human milk the phosphorus is almost all in organic com-

[1] Pfeiffer, Jahrb. f. Kinderheilkunde, **20**, also Maly's Jahresber., **13**; V. Adriance and J. Adriance, A Clinical Report of the Chemical Examination, etc., Archives of Pediatrics, 1897; Camerer and Söldner, Zeitschr. f. Biologie, **33** and **36**. In regard to the composition of woman's milk, see also Biel, Maly's Jahresber., **4**; Christenn, *ibid.*, **7**; Mendes de Leon, *ibid.*, **12**; Gerber, Bull. soc. chim., **23**; Tolmatscheff, Hoppe-Seyler's Med.-chem. Untersuch., 272.

bination. This does not agree with the results of SIKES who found on an average of only 42 per cent of the total P_2O_5 in organic combination. Because of the large amount of casein (and calcium phosphate) cow's milk is much richer in phosphorus than human milk. The relation P_2O_5:N, according to SCHLOSSMANN,[1] is equal to 1:5.4 in human milk and 1:2.7 in cow's milk. Woman's milk is poorer in mineral bodies, especially lime, and it contains only one-sixth of the quantity of lime as compared with cow's milk. The mineral constituents of human milk are better assimilated by the organism of the nursing child than those of cow's milk. Human milk is also claimed to be poorer in citric acid (SCHEIBE [2]), although this is not an essential difference.

Another difference between woman's milk and other varieties of milk is UMIKOFF's reaction, which seems to depend upon the quantitative composition, especially the relation between the milk-sugar, citric acid, lime, and iron (SIEBER [3]). This reaction consists in treating 5 cc. of woman's milk with 2.5 cc. ammonia (10 per cent) and heating to 60° C. for 15–20 minutes, when the mixture becomes violet-red. Cow's milk gives a yellowish-brown color when thus treated.

According to RUBNER woman's milk contains about 3 p. m. soaps, but this could not be substantiated by CAMERER and SÖLDNER. They conclude that woman's milk contains no soaps, or at least only very small amounts. They also found the quantity of urea nitrogen in woman's milk to be 0.11–0.12 p. m., although SCHÖNDORFF [4] found nearly twice this amount, namely, 0.23 p. m.

In regard to the quantity of *mineral bodies* in woman's milk we have the analyses of several investigators, especially of BUNGE (analyses *A* and *B*) and of SÖLDNER and CAMERER (analysis *C*).[5] BUNGE analyzed the milk of a woman, fourteen days after delivery, whose diet contained very little common salt for four days previous to the analysis (*A*), and again three days later after a daily addition of 30 grams of NaCl to the food (*B*). The figures are in 1000 parts of the milk:

	A	*B*	*C*
K_2O	0.780	0.703	0.884
Na_2O	0.232	0.257	0.357
CaO	0.328	0.343	0.378
MgO	0.064	0.065	0.053
Fe_2O_3	0.004	0.006	0.002
P_2O_5	0.473	0.469	0.310
Cl	0.438	0.445	0.591

The relation of the two bodies potassium and sodium to each other may, BUNGE believes, vary considerably (1.3–4.4 equivalents of potash

[1] Burow, Zeitschr. f. physiol. Chem., **30**; Koch, *ibid.*, **47**; Wittmaack, *ibid.*, **22**; Siegfried, *ibid.*, **22**; Nerking and Haensel, Bioch. Zeitschr., **13**; Valenti, Biochem. Centralbl., **4**; Schlossmann, Arch. f. Kinderheilkunde, **40**; Sikes, Journ. of Physiol., **34**.

[2] Maly's Jahresber., **21**.

[3] Zeitschr. f. physiol. Chem., **30**.

[4] Rubner, Zeitschr. f. Biologie, **36**; Camerer and Söldner, *ibid.*, **39**; Schöndorff, Pflüger's Arch., **81**.

[5] Bunge, Zeitschr. f. Biologie, **10**; Camerer and Söldner, *ibid.*, **39** and **44**.

to 1 of soda). By the addition of salt to the food, the quantity of sodium and chlorine in the milk increases, while the quantity of potassium decreases. De Lange found more Na than K in the milk at the beginning of lactation. Jolles and Friedjung found on an average 5.9 milligrams of iron per liter of woman's milk. Camerer and Söldner [1] find about the same amount, namely, 10–20 milligrams Fe_2O_3=3.5–7 milligrams iron in 1000 grams human milk.

The gases of woman's milk have been investigated by Külz.[2] He found 1.07–1.44 cc. of oxygen, 2.35–2.87 cc. of carbon dioxide, and 3.37–3.81 cc. of nitrogen in 100 cc. of milk.

The proper treatment of cow's milk by diluting it with water and by certain additions in order to render it a proper substitute for woman's milk in the nourishment of children cannot be determined before the difference in the protein bodies of these two kinds of milk has been completely studied.

The **colostrum** has a higher specific gravity, 1.040–1.060, a greater quantity of coagulable proteins, and a deeper yellow color than ordinary woman's milk. Even a few days after delivery the color becomes less yellow, the quantity of albumin less, and the number of colostrum-corpuscles diminishes.

We have the older analyses of Clemm [3] and the recent investigations of Pfeiffer, V. and J. Adriance, Camerer and Söldner on the changes in the composition of milk after delivery. It follows, as a unanimous result from these investigations, that the quantity of protein, which amounts to more the first two days, sometimes to more than 30 p. m. at first, rather quickly and then more generally diminishes as long as the lactation continues, so that in the third week it equals about 10–18 p. m. Like the protein substances, the mineral bodies also gradually decrease. The quantity of fat shows no regular or constant variation during lactation, while the lactose, especially according to the observations of V. and J. Adriance (120 analyses), increases rather quickly the first days and then only slowly until the end of lactation. The analyses of Pfeiffer, Camerer and Söldner also show an increase in the quantity of milk-sugar.

The two mammary glands of the same woman may yield somewhat different milk, as shown by Sourdat and later by Brunner.[4] Likewise the different portions of milk from the same milking may have varying composition. The first portions are always poorer in fat.

According to l'Héritier and to Vernois and Becquerel, the milk of blondes

[1] De Lange, Maly's Jahresber., 27; Jolles and Friedjung, Arch. f. exp. Path. u. Pharm., 46; Camerer and Söldner, Zeitschr. f. Biologie, 46.

[2] Zeitschr. f. Biologie, 32.

[3] See Hoppe-Seyler, Physiol. Chem., 734.

[4] Sourdat, Compt. rend., 71; Brunner, Pflüger's Arch., 7.

contains less casein than that of brunettes, a difference which TOLMATSCHEFF [1] could not substantiate. Women of delicate constitutions yield a milk richer in solids, especially in casein, than women with strong constitutions (V. and B.).

According to VERNOIS and BECQUEREL, the age of the woman has an effect on the composition of the milk, so that we find a greater quantity of proteins and fat in women 15–20 years old and a smaller quantity of sugar. The smallest quantity of proteins and the greatest quantity of sugar are found at 20 or from 25 to 30 years of age. VERNOIS and BECQUEREL, consider that the milk with the first-born is richer in water—with a proportionate diminution of casein, sugar, and fat—than after several deliveries.

The influence of menstruation seems to slightly diminish the milk-sugar and to considerably increase the fat and casein (VERNOIS and BECQUEREL).

Witch's milk is the secretion of the mammary glands of new-born children of both sexes immediately after birth. This secretion has from a qualitative standpoint the same constitution as milk, but may show important differences and variations from a quantitative point of view. SCHLOSSBERGER and HAUFF, GUBLER and QUEVENNE, and v. GENSER,[2] have made analyses of this milk and give the following results: 10.5–28 p. m. proteins, 8.2–14.6 p. m. fat, and 9–60 p. m. sugar.

As milk is the only form of nourishment during a certain period of the life of man and mammals, it must contain all the nutriment necessary for life. This fact is shown by the milk containing representatives of the three chief groups of organic nutritive substances—proteins, carbohydrates, and fat, and the last two groups can here also in part mutually substitute each other. Besides this all milk seems to contain, without doubt, also some lecithin and nucleon. The mineral bodies in milk must also occur in proper proportions, and on this point the experiments of BUNGE on dogs are of special interest. He found that the mineral bodies of the milk occur in about the same relative proportion as they do in the body of the sucking animal. BUNGE [3] found in 1000 parts of the ash the following results (*A* represents results from the new-born dog, and *B* the milk from the bitch):

	A	*B*
K_2O	114.2	149.8
Na_2O	106.4	88.0
CaO	295.2	272.4
MgO	18.2	15.4
Fe_2O_3	7.2	1.2
P_2O_5	394.2	342.2
Cl	83.5	169.0

BUNGE explains the fact that the milk-ash is richer in potash and poorer in soda than the new-born animal by saying that in the growing animal the ash of the muscles rich in potash relatively increases and the cartilage rich in soda relatively decreases. In regard to the amount

[1] l'Héritier, cited from Hoppe-Seyler, Physiol. Chem., 738; Vernois and Becquerel, Du lait chez la femme dans l'état de santé, etc. (Paris, 1853); Tolmatscheff, Hoppe-Seyler, Med.-chem. Untersuch., 272.

[2] Schlossberger and Hauff, Annal. d. Chem. u. Pharm., **96**; Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 723; v. Genser, *ibid.*

[3] Zeitschr. f. physiol. Chem., **13**.

of iron we find an unexpected condition, the ash of the new-born animal containing six times as much as the milk-ash. This condition BUNGE explains by the fact founded on his and ZALESKY's experiments, that the quantity of iron in the entire organism is highest at birth. The new-born has therefore its own supply of iron for the growth of its organs even at birth.

The investigations of HUGOUNENQ, DE LANGE, CAMERER and SÖLDNER [1] have shown that in man the conditions are different from those in animals, as the ash of the child has an entirely different composition as compared with the milk. As an example the following analyses are given (of CAMERER and SÖLDNER). (*A*, the ash of the sucking infant, and *B*, the ash of the milk.) The results are in 1000 parts of the ash.

	A	*B*
K_2O	78	314
Na_2O	91	119
CaO	361	164
MgO	9	26
Fe_2O_3	8	6
P_2O_5	389	135
Cl	77	200

We cannot therefore state as a definite fact that the composition of the ash of the sucking young and the ash of the corresponding milk coincide. BUNGE [2] nevertheless claims that the composition of the ash of the sucking young of various mammals is nearly the same, but that the ash of the milk differs from the ash of the young in so far as the slower the young grows the richer it is in alkali chlorides and relatively poorer in phosphates and lime-salts. The constituents of the ash have two functions to perform, namely, the building up of the tissues and secondly the preparation of the excreta, especially the urine. The faster the young grows the more is the first in evidence, while the slower it develops, the more prominent is the second.

The quantity of mineral bodies in the milk, and especially the amount of lime and phosphoric acid, as shown by BUNGE and PRÖSCHER and PAGÈS, stands in close relation to the rapidity of growth, because the amount of these mineral constituents in the milk is greater in animals which grow and develop quickly than in those which grow only slowly. A similar relation also exists, as shown by the researches of PRÖSCHER, and especially of ABDERHALDEN,[3] between the quantity of protein in the milk and the rapidity of development of the sucking young. The amount of protein is greater in the milk the quicker the animal develops.

[1] Hugounenq, Compt. rend., **128**; de Lange, Zeitschr. f. Biologie, **40**; Camerer and Söldner, *ibid.*, **39**, **40**, and **44**.

[2] Bunge, "Die zunehmende Unfähigkeit der Frauen ihre Kinder zu stillen," München, 1900, cited by Camerer, Zeitschr. f. Biologie, **40**.

[3] Pröscher, Zeitschr. f. physiol. Chem., **24**; Abderhalden, *ibid.*, **27**; Pagès, Arch. de Physiol. (5), **7**.

The *influence of the food* on the composition of the milk is of interest from many points of view and has been the subject of many investigations. From these we learn that in human beings as well as in animals an insufficient diet decreases the quantity of milk and the quantity of solids, while abundant food increases both. From the observations of DECAISNE [1] on nursing women during the siege of Paris in 1871, the amount of casein, fat, sugar, and salts, but especially the fat, was found to decrease with insufficient food, while the quantity of lactalbumin was found to be somewhat increased. Food rich in proteins increases the quantity of milk, and also the solids contained, especially the fat, according to most reports. The quantity of sugar in woman's milk is found by certain investigators to be increased after food rich in proteins, while others claim it is diminished. A diet rich in fat may, as the researches of SOXHLET and many others [2] have shown, cause a marked increase in the fat of the milk when the fat partaken is in a readily digestible and assimilable form. The presence of large quantities of carbohydrates in the food seems to cause no constant, direct action on the quantity of the milk constituents.[3] From feeding experiments with different foods we come to the conclusion that the character of the food is of comparatively little influence, while the race and other conditions play an important role. Watery food gives a milk containing an excess of water and having little value. In the milk from cows which were fed on distillers' grain COMMAILLE [4] found 906.5 p. m. water, 26.4 p. m. casein, 4.3 p. m. albumin, 18.2 p. m. fat, and 33.8 p. m. sugar. Such milk has sometimes a peculiar sharp after-taste, although not always.[5]

Chemistry of Milk-secretion. That the constituents which occur actually dissolved in milk pass into the secretion and not alone by filtration or diffusion, but more likely are secreted by a specific secretory activity of the granular elements, is shown by the fact that milk-sugar, which is not found in the blood, is to all appearances formed in the glands themselves. A further proof lies in the fact that the lactalbumin is not identical with seralbumin; and lastly, as BUNGE [6] has shown, the mineral bodies

[1] Cited from Hoppe-Seyler, l. c., 739.

[2] See Maly's Jahresber., **26**. See also Basch, Ergebnisse der Physiologie, **2**, Abt. 1.

[3] In regard to the literature on the action of various foods on woman's milk, see Zalesky, "Ueber die Einwirkung der Nahrung auf die Zusammensetzung und Nahrhaftigkeit der Frauenmilch," Berlin. klin. Wochenschr., 1888, which also contains the literature on the importance of diet on the composition of other kinds of milk. In regard to the extensive literature on the influence of various foods on the milk production of animals, see König, Chem. d. menschl. Nahrungs und Genussmittel, 3. Aufl., **1**, 298. See also Maly's Jahresber., **29**, **37**, and Morgen, Beger and Fingerling, Landw. Versuchsst., **61**, and Raudnitz, Monatschr. f. Kinderheilk.

[4] Cited from König, **2**, 235.

[5] See Beck, Maly's Jahresber., **25**.

[6] Lehrbuch d. physiol. und pathol. Chem., 3. Aufl., 93.

secreted by the milk are in quite different proportions from those in the blood-serum.

Little is known in regard to the formation and secretion of the specific constituents of milk. The older theory, that the casein was produced from the lactalbumin by the action of an enzyme, is incorrect, and probably originated from mistaking an alkali albuminate for casein. Better founded is the theory that the casein originates from the protoplasm of the gland-cells. There does not seem to be any doubt that the protoplasm of the cells takes part in the secretion in such a manner that it becomes itself a constituent of the secretion, and this also agrees with HEIDENHAIN'S[1] views. According to BASCH'S researches, the casein is formed in the mammary gland by the nucleic acid of the nucleus being set free and uniting intra-alveolar with the transudated serum, thus forming a nucleoalbumin, the casein. The untenableness of this view has been shown by LÖBISCH, and the investigations of HILDEBRANDT[2] upon the proteolytic enzyme of the mammary gland, and the autolysis of the gland have not given any clue as to the mode of formation of casein. There is no doubt that it is formed in the mammary glands by a synthesis. The findings of MANDEL[3] that the hydrolytic cleavage products of the nucleoprotein from the mammary glands occur approximately quantatively in the same proportions as in casein, are important in this connection.

That the milk-fat is produced by a formation of fat in the protoplasm, and that the fat-globules are set free by their destruction, is a generally admitted opinion, which, however, does not exclude the possibility that the fat is in part taken up by the glands from the blood and eliminated with its secretion. That the fats of the food can pass into the milk follows from the investigations of WINTERNITZ, as he has been able to detect the passage of iodized fats in the milk. JANTZEN has shown that after feeding iodized casein, the milk-fat of goats contained a little iodine, which indicates that the iodized milk-fat could also have a different origin. As a contamination of the casein fed with iodized fat was not excluded in these experiments, they do not seem to modify the proof of the investigations of WINTERNITZ and others (CASPARI, PARASCHTSCHUK[4]). The abundant quantities of iodized fat which were eliminated with the milk in these cases without doubt depend, at least in great part, upon the iodized fat of the food, hence it cannot be said that all of the milk-fat

[1] Hermann's Handbuch, **5**, Teil 1, 380.

[2] Basch, Jahrb. f. Kinderheilkunde, 1898; Hildebrandt, Hofmeister's Beiträge, **5**; Löbisch, *ibid.*, 8.

[3] Bioch. Zeitschr., **22**.

[4] Winternitz, Zeitschr. f. physiol. Chem., **24**; Jantzen, Centralbl. f. Physiol., **15**; Caspari, Arch. f. (Anat. u.) Physiol., 1899, Supplbd. and Zeitschr. f. Biologie, **46**; Paraschtschuk, Chem. Centralbl., 1903, **1**.

containing iodine was unchanged iodized fat of the food. The investigations of SPAMPANI and DADDI, PARASCHTSCHUK, GOGITIDSE and others on the passage of foreign fats into the milk also indicate the passage of the fat of the food into the milk, although we are still uncertain on this point. According to SOXHLET the fat of the food does not pass into the milk directly, but is destroyed in place of the body-fat, which then becomes available and is, as it were, pushed into the milk. HENRIQUES and HANSEN could not detect any mentionable quantity of linseed-oil in the milk after feeding with this oil; the milk-fat was not normal, but had a higher iodine equivalent and a higher melting-point, from which they also concluded that a transformation of the food-fat in the glandular cells is possible. The results of the experiments of GOGITIDSE [1] with soaps also indicate that the mammary glands have the property of forming fats by synthesis from their components. As a formation of fat from carbohydrates in the animal organism is at the present day considered as positively proven, it is likewise possible that the milk-glands also produce fats from the carbohydrates brought to them by the blood. It is a well-known fact that an animal gives off for a long time, daily, considerably more fat in the milk than it receives as food, and this proves that at least a part of the fat secreted by the milk is produced from proteins or carbohydrates, or perhaps from both. The question as to how far this fat is produced directly in the milk-glands, or from other organs and tissues, and brought to the gland by means of the blood, cannot be decided.

The origin of milk-sugar is not known. MÜNTZ calls attention to the fact that a number of very widely diffused bodies in the vegetable kingdom—vegetable mucilage, gums, pectin bodies—yield galactose as a product of decomposition, and he believes, therefore, that milk-sugar may be formed in herbivora by a synthesis from dextrose and galactose. This origin of milk-sugar does not apply to carnivora, as they produce milk-sugar when fed on food consisting entirely of lean meat. The observations of BERT and THIERFELDER [2] that a mother-substance of the milk-sugar, a saccharogen, occurs in the glands, does not explain the formation of milk-sugar, as the nature of this mother-substance is still unknown. As the animal body has undoubtedly the power of converting one variety of sugar into another, the origin of the milk-sugar can be sought simply in the dextrose introduced as food or formed in the body. Certain observations indicate such an origin, among others those of PORCHER, who found that dextrose appeared in the urine after delivery when the mammary glands of the goat had previously been

[1] Spampani and Daddi, Maly's Jahresber., **26**; Henriques and Hansen, *ibid.*, **29**; Gogitidse, Zeitschr. f. Biologie, **45**, **46**, and **47**. See also Basch, Ergebnisse d. Physiol., **2**, Abt. 1.

[2] Müntz, Compt. rend., **102**; Bert and Thierfelder, footnote 1, p. 611.

extirpated. This glycosuria is explained simply by the fact that the lactose-forming action of the gland was removed at the time of delivery, when large amounts of dextrose were produced, but it must not be forgotten that MARSHALL and KIRKNESS found with similar experiments upon guinea-pigs no passage of sugar into the urine. The experiments of KAUFMANN and MAGNE [1] upon cows indicate a formation of lactose from dextrose. They found that during secretion the glands took sugar from the blood, so that the venous gland-blood was poorer in sugar than otherwise.

The passage of foreign substances into the milk stands in close connection with the chemical processes of milk secretion.

It is a well-known fact that milk acquires a foreign taste from the food of the animal, which is in itself a proof that foreign bodies pass into the milk. This fact becomes of special importance in reference to such injurious substances as may be introduced into the organism of the nursing child by means of the milk.

Among these substances may be mentioned opium and morphine, which after large doses pass into the milk and act on the child. Alcohol may also pass into the milk, but probably not in such quantities as to have any direct action on the nursing child.[2] Alcohol is claimed to have been detected in the milk after feeding cows with brewer's grains.

Among inorganic bodies, iodine, arsenic, bismuth, antimony, zinc, lead, mercury, and iron have been found in milk. In icterus neither bile-acids nor bile-pigments pass into the milk.

Under diseased conditions no constant change has been found in woman's milk. In isolated cases. SCHLOSSBERGER, JOLY and FILHOL [3] have indeed observed a markedly abnormal composition, but no positive conclusion can be derived therefrom.

The changes in cow's milk in disease have been little studied. In tuberculosis of the udder STORCH [4] found tubercle bacilli in the milk, and he also noted that the milk became more and more diluted, during the disease, with a serous liquid similar to blood-serum, so that the glands finally, instead of yielding milk, gave only blood-serum or a serous fluid. HUSSON [5] found that milk from murrain cows contained more proteins but considerably less fat and (in severe cases) less sugar than normal milk.

The milk may be blue or red in color, due to the development of microorganisms.

The formation of concrements in the exit-passages of the cow's udder is often observed. These consist chiefly of calcium carbonate, or of carbonate and phosphate with only a small amount of organic substances.

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[1] Porcher, Compt. rend., **138** and **141**; Marshall and Kirkness, Bioch. Journ., **2**; Kaufmann and Magne, Compt. rend., **143**.

[2] See Klingemann, Virchow's Arch., **126**, and Rosemann, Pflüger's Arch., **78**.

[3] Schlossberger, Annal. d. Chem. u. Pharm., **96**; Joly and Filhol, cited from v. Gorup-Besanez, Lehrb., 4. Aufl., 438.

[4] See Bang, Om Tuberkulose i Koens Yver og om tuberkulös Mälk, Nord. med. Arkiv, **16**, and also Maly's Jahresber., **14**, 170; Storch, Maly's Jahresber., **14**.

[5] Compt. rend., **73**.

CHAPTER XV.

URINE.

URINE is the most important excretion of the animal organism; it is the means of eliminating the nitrogenous metabolic products, also the water and the soluble mineral substances; and in many cases it furnishes important data relative to the metabolism, quantitatively by its variation, and qualitatively by the appearance of foreign bodies in the excretion. Moreover, in many cases we are able from the chemical or morphological constituents which the urine abstracts from the kidneys, ureter, bladder, and urethra to judge of the condition of these organs; and lastly urinary analysis affords an excellent means of deciding the question as to how certain medicinal agents or other foreign substances introduced into the organism are absorbed and chemically changed. In this respect, urinary analysis has furnished very important particulars especially in regard to the nature of the chemical processes taking place within the organism, and it is therefore not only an important aid to the physician in diagnosis, but it is also of the greatest importance to the toxicologist and the physiological chemist.

In studying the secretions and excretions the relation must be sought between the chemical structure of the secreting organ and the chemical composition of its secreted products. Investigations with respect to the kidneys and the urine have led to very few results from this standpoint. Although the anatomical relation of the kidneys has been carefully studied, their chemical composition has not been the subject of thorough analytical research. In cases in which a chemical investigation of the kidneys has been undertaken, it has been in general only of the organ as such, and not of the different anatomical parts. An enumeration of the chemical constituents of the kidneys known at the present time can, therefore, only have a secondary value.

In the kidneys we find proteins of different kinds. According to HALLIBURTON the kidneys do not contain any albumin, but only a *globulin* and a *nucleoprotein*. The globulin coagulates at about 52° C., and the nucleoprotein contains 0.37 per cent phosphorus. LIEBERMANN claims that the kidneys contain a *lecithalbumin*, and he ascribes to this body a special importance in the secretion of acid urines. The kidneys also contain, according to LÖNNBERG, a *mucin-like substance*.

This substance yields no reducing body on boiling with acids, and belongs chiefly to the papillæ, and is, this author says, a nucleoalbumin (nucleoproteid?). The cortical substance is richer in another nucleoalbumin (nucleoproteid) unlike mucin. It has not been decided what relation this last substance bears to HALLIBURTON'S nucleoprotein. The nucleic acid obtained by MANDEL and LEVENE from beef kidneys yielded guanine, adenine, thymine, and cytosine on cleavage. According to MÖRNER [1] *chondroitin-sulphuric acid* occurs as traces. MANDEL and LEVENE [2] have also obtained *glucothionic acid* from the kidneys. *Fat* occurs only in very small amounts in the cells of the convoluted tubules. FRÄNKEL and NOGUEIRA [3] found a cephalin-like substance, a triamido-diphosphatide and a diamino-monophosphatide. Among the extractive bodies of the kidneys one finds *purine bases*, also *urea*, *uric acid* (traces), *glycogen*, *leucine*, *inosite*, *taurine*, and *cystine* (in ox-kidneys). The quantitative analyses of the kidneys thus far made possess little interest. OIDTMANN [4] found 810.94 p. m. water, 179.16 p. m. organic and 0.99 p. m. inorganic substance in the kidney of an old woman.

The fluid collected under pathological conditions, as in hydronephrosis, is thin with a variable but generally low specific gravity. Usually it is straw-yellow or paler in color, and sometimes colorless. Most frequently it is clear, or only faintly cloudy from white blood-corpuscles and epithelium-cells; in a few cases it is so rich in form-elements that it appears like pus. Protein generally occurs in small amounts; occasionally it is entirely absent, but in a few rare cases the amount is nearly as large as in the blood-serum. Urea occurs sometimes in considerable amounts when the parenchyma of the kidneys is only in part atrophied; in complete atrophy the urea may be entirely absent.

I. PHYSICAL PROPERTIES OF URINE.

Consistency, Transparency, Odor, and Taste of Urine. Under physiological conditions urine is a thin liquid and gives, when shaken with air, a froth which quickly subsides. Human urine, or urine from carnivora, which is habitually acid, appears clear and transparent, often faintly fluorescent, immediately after voiding. When allowed to stand for a little while human urine shows a light cloud (*nubecula*), which consists of the so-called "mucus," and generally also contains a few epithelium cells, mucus-corpuscles, and urate-granules. The presence of a larger quantity of urates renders the urine cloudy, and a clay-yellow,

[1] Halliburton, Journ. of Physiol., **13**, Suppl., and **18**; Liebermann, Pflüger's Arch., **50** and **54**; Lönnberg, see Maly's Jahresber., **20**; Mandel and Levene, Zeitschr. f. physiol. Chem., **47**; Mörner, Skand. Arch. f. Physiol., **6**.

[2] Zeitschr. f. physiol. Chem., **45**. See also Mandel and Neuberg, Bioch. Zeitschr., **13**.

[3] Bioch. Zeitschr., **16**. See also Dunham, Zeitschr. f. physiol. Chem., **64**.

[4] Cited from v. Gorup-Besanez, Lehrb., 4. Aufl., 732.

yellowish-brown, rose-colored, or often brick-red precipitate (*sedimentum lateritium*) settles on cooling, because of the greater insolubility of the urates at the ordinary temperature than at the temperature of the body. This cloudiness disappears on gently warming. In new-born infants the cloudiness of the urine during the first 4–5 days is due to epithelium, mucus-corpuscles, uric acid, and urates. The urine of herbivora, which is habitually neutral or alkaline in reaction, is very cloudy on account of the carbonates of the alkaline earths present. Human urine may sometimes be alkaline under physiological conditions. In this case it is cloudy, due to the earthy phosphates, and this cloudiness does not disappear on warming, differing in this respect from the *sedimentum lateritium*. Urine has a salty and faintly bitter taste produced by sodium chloride and urea. The odor of urine is peculiarly aromatic; the bodies which produce this odor are unknown.

The **color** of urine is normally pale yellow when the specific gravity is 1.020. The color otherwise depends on the concentration of the urine and varies from pale straw-yellow, when the urine contains small amounts of solids, to a dark reddish yellow or reddish brown in stronger concentration. As a rule the intensity of the color corresponds to the concentration, but under pathological conditions exceptions occur such as are found in diabetic urine, which contains a large amount of solids and has a high specific gravity and a pale-yellow color.

The **reaction** of urine depends essentially upon the composition of the food. The carnivora, as a rule, void an acid, the herbivora, a neutral or alkaline urine. If a carnivore is put upon a vegetable diet, its urine may become less acid or neutral, while the reverse occurs when an herbivore is starved, that is, when it lives upon its own flesh, as then the urine voided is acid.

The urine of a healthy man on a mixed diet has an *acid reaction*, and the sum of the acid equivalents is greater than the sum of the basic equivalents. This depends upon the fact that in the physiological combustion of neutral substances (proteins and others) within the organism, acids are produced, chiefly sulphuric acid, but also phosphoric and organic acids, such as hippuric, uric, and oxalic acids, aromatic oxyacids, oxyproteic acids[1] and others. From this it follows that the acid reaction is not due to one acid alone. The various acids take part in the acid reaction in proportion to their dissociation, since, according to the ion theory, the acid reaction of a mixture is dependent upon the number of hydrogen ions present. Hence the theory that the acidity is due entirely to dihydrogen phosphate is incorrect although this salt takes such a great part in the acid reaction that its quantity is often taken as a measure of the degree of acidity of the urine.

[1] See St. Kozlowski, Bull. Acad. d. scien. de Cracovie, Jan., 1909, 37.

The composition of the food is not the only influence which affects the degree of acidity of human urine. For example, after taking food at the beginning of digestion, when a larger amount of gastric juice containing hydrochloric acid is secreted, the urine may be neutral or even alkaline.[1] As to the time of the appearance of the maximum and minimum of acidity, the various investigators do not agree, which may in part be explained by the varying individuality and conditions of life of the persons investigated. It has not infrequently been observed that perfectly healthy persons in the morning void a neutral or alkaline urine which is cloudy from earthy phosphates. The effect of muscular activity on the acidity of urine has not been positively determined. According to HOFFMANN, RINGSTEDT, ODDI, and TARULLI and VOZÁRIK muscular work raises the degree of acidity, but ADUCCO[2] claims that it decreases it. Abundant perspiration reduces the acidity (HOFFMANN).

In man and especially in carnivora it seems that the degree of acidity of the urine cannot be increased above a certain point, even though mineral acids or organic acids which are burnt up with difficulty are ingested in large quantities. When the supply of carbonates of the fixed alkalies stored up in the organism for this purpose is not sufficient to combine with the excess of acid, then ammonia is split off from the proteins or their decomposition products, and this excess of acid combines therewith, forming ammonium salts, which pass into the urine. In herbivora such a combination of the excess of acid with ammonia seems not to take place, or not to the same extent, and therefore herbivora soon die when acids are given. This is true at least for rabbits, while according to BAER[3] this power of increasing the elimination of ammonia exists also in the goat, monkey, and pig, hence no definite difference in this regard exists between herbivora and carnivora. The differences which have been observed are, according to EPPINGER, not of a special kind, and they may be caused, he says, from a different amount of protein in the food which yields ammonia. One can by food rich in protein make herbivora (also rabbits) resistant toward the introduction of acid, while dogs with food poor in protein behave like rabbits. The question as to the action of acids upon the elimination of fixed alkalies by the urine and the removal of these from the tissues seems to be rather complicated as, according to STAAL, in rabbits the quantity of sodium in the subcutaneous connective tissue is not diminished after the continuous introduction of acid for several days, but is rather

[1] Contradictory statements are found in Linossier, Maly's Jahresber., **27**.

[2] Hoffmann, see Maly's Jasresber., **14**; Ringstedt, *ibid.*, **20**; Oddi and Tarulli, *ibid.*, **24**; Aducco, *ibid.*, **17**; Vozárik, Pflüger's Arch., **111**.

[3] See Winterberg, Zeitschr. f. physiol. Chem., **25**, and J. Baer, Arch. f. exp. Path. u. Pharm., **54**.

increased. It must not be overlooked that, as A. Loewy[1] has found, the sensitiveness of different individuals toward the action of acid varies considerably.

Although one cannot raise the degree of acidity of the urine above a certain limit by the introduction of acid, still it may be easily diminished, so that the reaction becomes neutral or alkaline. This occurs after the taking of carbonates of the fixed alkalies or of such alkali salts of vegetable acids—citric acid, and malic acid—as are easily burnt into carbonates in the organism. Under pathological conditions, as in the absorption of alkaline transudates, or the alkaline fermentation within the bladder, the urine may become alkaline.

A urine with an alkaline reaction caused by fixed alkalies has a very different diagnostic value from one whose alkaline reaction is caused by the presence of ammonium carbonate. In the latter case we have to deal with a decomposition of the urea of the urine by the action of microorganisms.

If one wishes to determine whether the alkaline reaction of the urine is due to ammonia or to fixed alkalies, a piece of red litmus paper is dipped into the urine and allowed to dry exposed to the air or to a gentle heat. If the alkaline reaction is due to ammonia, the paper becomes red again; but if it is caused by fixed alkalies, it remains blue.

Determination of the Acidity. As the quantity of phosphoric acid present as dihydrogen salt, as above stated, cannot be used as a measure of the acidity, none of the older methods suggested for the estimation of this portion of the phosphoric acid is suited for acidity determinations. We now determine the acidity simply by acidimetric methods, titrating with N/10 caustic alkali, using phenolphthalein as an indicator (Naegeli, Höber, Folin). On account of the color of the urine and the presence of ammonium salts and alkaline earths, this method cannot yield entirely exact results. The greatest error is due to the alkaline earths, which, on titration with caustic alkali, precipitate as earthy phosphates in variable amounts and of variable composition. This error can be prevented, according to Folin, by the addition of neutral potassium oxalate, which precipitates the lime, and in this way the disturbing action of the ammonium salts is also inhibited. Perfectly accurate results are not obtained by this method, but it is the best of those which have been suggested.

It is performed as follows: 25 cc. of urine are placed in an Erlenmeyer flask (about 200 cc. capacity), treated with 1–2 drops of ½-per cent phenolphthalein solution, and shaken with 15–20 grams of powdered

[1] Eppinger, Zeitschr. f. exp. Path. u. Therap., **3**; with Tedesko, Bioch. Zeitschr., **16**; Staal, Zeitschr. f. physiol. Chem., **58**; A. Loewy, Centralbl. f. Physiol., **20**, 337.

potassium oxalate and immediately titrated with N/10 caustic soda with constant shaking until a pronounced pale-rose color appears. Vozárik [1] titrates the diluted urine without the addition of oxalate and uses phenolphthalein as indicator.

The acidity, as determined by titration, varies considerably under physiological conditions, but calculated as hydrochloric acid it amounts in man to about 1.5–2.3 grams in the twenty-four hours.

By titration we learn the amount of hydrogen present which can be substituted by a metal, i.e., the acidity in the ordinary older sense, but not the true acidity, the ion acidity, which is given by the concentration of the hydrogen ions of the urine. For similar reasons, as previously indicated in treating of the alkalinity of the blood-serum (page 264), the ion acidity cannot be determined by titration, while it can be determined according to the principle of the electrometric gas-chain method as there given. Such estimations have been made by v. Rhorer and by Höber.[2] For normal urine v. Rhorer found as a minimum 4×10^{-7}, as a maximum 76×10^{-7}, and as an average 30×10^{-7}. Höber found 4.7×10^{-7}, 100×10^{-7}, and 49×10^{-7}, respectively. On an average the urine therefore contains 30–50 grams of hydrogen ions in 10 million liters, and as in the same quantity of purest water there is contained in round numbers 1 gram of hydrogen ions, the urine contains, therefore, 30–50 times as many hydrogen ions as the water. From Höber's investigations it also follows that no direct relation exists between the titration acidity and the ion acidity, and that the extent of these two acidities may be independent of each other.

The **osmotic pressure** of the urine varies considerably even under physiological conditions. The limit for the freezing-point depression has been found by a number of investigators to be $\Delta=0.87$–$2.71°$ C.[3] After partaking of considerable water it may be markedly lower, and on diminished supply of water it may be considerably higher.

According to Bugarsky a certain relation exists between the freezing-point depression and the specific gravity, namely, $\frac{\Delta}{s-1}=\text{constant}=75$. This equation, where s represents the specific gravity, has no general application, and according to Steyrer [4] is only approximate for normal urines. The validity of the relation found by Bugarsky between the electrical conductivity and the ash content of the urine, seems also to require further proof.

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[1] In regard to the degree of acidity and its estimation see Naegeli, Zeitschr. f. physiol. Chem., **30**; Höber, Hofmeister's Beiträge, **3**; Folin, Amer. Journ. of Physiol., **9**; Vozárik, l. c.; de Jager, Zeitschr. f. physiol. Chem., **55**, and Ringer, *ibid.*, **60**.

[2] v. Rhorer, Pflüger's Arch., **86**; Höber, l. c. See also Jolles, Bioch. Zeitschr., **13**.

[3] See Strauss, Zeitschr. f. klin. Med., **47**.

[4] Bugarsky, Pflüger's Arch., **68**; Steyrer, Hofmeister's Beiträge, **2**.

The specific gravity of urine, which is dependent upon the relation existing between the quantity of water secreted and the solid urinary constituents, especially the urea and sodium chloride, may vary considerably, but is generally 1.017–1.020. After drinking large quantities of water it may fall to 1.002, while after profuse perspiration or after drinking very little water it may rise to 1.035–1.040. In new-born infants the specific gravity is low, 1.007–1.005. The determination of the specific gravity is an important means of learning the average amount of solids eliminated from the organism in the urine, and on this account the determination becomes of true value only when at the same time the quantity of urine voided in a given time is determined. The different portions of urine voided in the course of the twenty-four hours are collected, mixed together, the total quantity measured, and then the specific gravity taken.

The *determination of the specific gravity* is most accurately obtained with the pycnometer. For ordinary cases the specific gravity may be determined with sufficient accuracy by means of areometers. The areometers found in the trade, or *urinometers*, are graduated from 1.000 to 1.040; for exact observations it is better to use two urinometers, one graduated from 1.000 to 1.020, and the other from 1.020 to 1.040.

To determine the specific gravity of urine, if necessary filter the urine, or if it contains a urate sediment, first dissolve it by gentle heat, then pour the clear urine into a dry cylinder, avoiding the formation of froth. Air bubbles or froth, when present, must be removed with a glass rod or filter-paper. The cylinder, which should be about four-fifths full, must be wide enough to allow the urinometer to swim freely in the liquid without touching the sides. The cylinder and urinometer should both be dry or previously washed with the urine. On reading, the eye is brought on a level with the lower meniscus—which occurs when the surface of the liquid and the lower limb of the meniscus coincide; the reading is then made from the point where this curved line coincides with the scale of the urinometer. If the eye is not in the same horizontal plane with the convex line of the meniscus, but is too high or too low, the surface of the liquid assumes the shape of an ellipse, and the reading in this position is incorrect. Before reading, press the urinometer gently down into the liquid and then allow it to rise, and wait until it is at rest.

Each urinometer is graduated for a certain temperature, which, at least in the case of the better ones, is marked on the instrument. If the urine is not at the proper temperature, the following corrections must be made: For every three degrees above the normal temperature one unit of the last order is added to the reading, and for every three degrees below the normal temperature one unit (as above) is subtracted from the specific gravity observed. For example, when a urinometer graduated for 15° C. shows a specific gravity of 1.017 at 24° C., then the specific gravity at 15° C. = 1.017 + 0.003 = 1.020.

When great exactitude is required, as, for instance, a determination to the fourth decimal point, we make use of a urinometer constructed

by LOHNSTEIN.[1] JOLLES[2] has also devised a small urinometer for the determination of the specific gravity of small amounts of urine, 20–25 cc. The specific gravity may also be determined by the WESTPHAL hydrostatic balance.

II. ORGANIC PHYSIOLOGICAL CONSTITUENTS OF URINE.

Urea, $\overset{+}{U}r$, $CON_2H_4=CO\begin{matrix}NH_2\\NH_2\end{matrix}$, has been synthetically prepared in several ways, especially, as WÖHLER showed in 1828, by the metameric transformation of ammonium isocyanate: $CO.N.NH_4=CO(NH_2)_2$. It is also produced by the decomposition or oxidation of certain bodies found in the animal organism, such as purine bodies, creatine, arginine, other amino-acids, and others.

Urea is found most abundantly in the urine of carnivora and man, but in smaller quantities in that of herbivora. In carnivora (dog) the urea nitrogen by abundant protein feeding may amount to 97–98 per cent of the total nitrogen of the urine (SCHÖNDORFF[3]). The quantity in human urine is ordinarily 20–30 p. m. It has also been found in small quantities in the urine of amphibians, fishes, and certain birds. Urea occurs in the perspiration in small quantities, and as traces in the blood and in most of the animal fluids. It also occurs in rather large quantities in the blood, liver, muscle,[4] and bile[5] of sharks. Urea is also found in certain tissues and organs of mammals, especially in the liver, spleen, muscles and others, although only in small amounts. Under pathological conditions, as in obstructed excretion, urea may appear to a considerable extent in the animal fluids and tissues.

The quantity of urea which is voided in twenty-four hours on a mixed diet is in a grown man about 30 grams, in women somewhat less. While children void less, the excretion relative to their body weight is greater than in grown persons. The physiological significance of urea lies in the fact that this body forms in man and carnivora, from a quantitative standpoint, the most important nitrogenous end-product of the metabolism of protein bodies. On this account the elimination of urea varies to a great extent with the catabolism of the protein, and above all with the quantity of absorbable proteins in the food ingested. The elimination of urea is greatest after an exclusive meat diet, and lowest, indeed less than during starvation, after the consumption of non-nitrogenous substances, since these diminish the metabolism of the proteins of the body.

[1] Pflüger's Arch., **59**; Chem. Centralbl., 1895, **1**, and 1896, **2**.

[2] Wien. med. Presse, 1897, No. 8.

[3] Pfluger's Arch., **117**.

[4] v. Schroeder, Zeitschr. f. physiol. Chem., **14**.

[5] Hammarsten, *ibid.*, **24**.

If the consumption of the proteins of the body is increased, then the elimination of nitrogen is correspondingly increased. This is found to be the case in fevers, after poisoning with arsenic, antimony, phosphorus, and other protoplasmic poisons, and when there is a diminished supply of oxygen—as in severe and continuous dyspnœa, poisoning with carbon monoxide, hemorrhage, etc. In these cases it used to be considered that the rise in the excretion of nitrogen was due to an increased elimination of urea, because no exact difference was made between the quantity of urea and of total nitrogen in the urine. Recent researches have conclusively demonstrated the untrustworthiness of these observations. Since PFLÜGER and BOHLAND have shown that 16 per cent of the total nitrogen of the urine exists under physiological conditions in other compounds, not urea, attention has been called to the relation of the different nitrogenous constituents of the urine to each other, and it has been found, under pathological conditions, that this relation may vary considerably, especially in regard to the urea. We have numerous determinations by different investigators, such as BOHLAND, E. SCHULTZE, CAMERER, VOGES, MÖRNER and SJÖQVIST, GUMLICH, BÖDTKER, FOLIN,[1] and others, on the relation of the different nitrogenous constituents to each other in the normal urine of adults. SJÖQVIST has made similar determinations on new-born babes from 1 to 7 days old. From all these analyses we obtain the following figures (A for adults and B for new-born babes). Of the total nitrogen there exists:

	A. Per Cent.	B. Per Cent.
Urea	84–91	73–76
Ammonia	2–5	7.8–9.6
Uric acid	1–3	3.0–8.5
Remaining nitrogeneous substances (extractives)	7–12	7.3–14.7

The variable relation between uric acid, ammonia, and urea nitrogen in children and adults is remarkable, since the urine of children is considerably richer in uric acid and ammonia, and considerably poorer in urea, than the urine of adults. A much larger number of analyses of children's urine is necessary to explain the division of the nitrogen therein. The absolute quantity of urea nitrogen in adults amounts to about 10–16 grams per day. In disease the proportion of the nitrogenous substances may be markedly changed, and a decrease in the quantity of urea and an increase in the quantity of ammonia have been

[1] Pflüger and Bohland, Pflüger's Arch., **38** and **43**; Bohland, *ibid.*, **43**; Schultze, *ibid.*, **45**; Camerer, Zeitschr. f. Biologie, **24**, **27**, and **28**; Voges, Ueber die Mischung der stickstoffhaltigen Bestandtheile im Harn, etc, (Inaug.-Diss. Berlin, 1982), cited from Maly's Jahresber., **22**; K. Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**. See also Sjöqvist, Nord. med. Arkiv., 1892, No. 36, and 1894, No. 10; Gumlich, Zeitschr. f. physiol. Chem., **17**; Bödtker, see Maly's Jahresber., **26**; Folin, Amer. Journ. of Physiol., **13**; Osterberg and Wolff, Journ. of biol. Chem., **3**; Haskins, *ibid.*, **2**.

observed in certain diseases of the liver. This will be considered in detail in connection with the formation of urea in the liver. It is natural that there should be a diminished formation of urea after a decrease in the ingestion of proteins or in a lowered catabolism. In diseases of the kidneys which disturb or destroy the integrity of the epithelium of the convoluted urinary tubules, the elimination of urea is considerably diminished.

Recently by means of PFAUNDLER'S [1] method, by precipitating the urine with phosphotungstic acid and closely studying the precipitate as well as the filtrate, it has been possible to learn further about the division of the nitrogen of the urine. We determine *a*, the total nitrogen; *b*, the nitrogen of the phosphotungstate precipitate; and *c*, the nitrogen in the filtrate from the phosphotungstate precipitate. This last contains the urea, hippuric acid, oxyproteic acids, and other bodies whose nitrogen is ordinarily designated as monamino-acid nitrogen. The urea nitrogen is especially determined. The bodies precipitated by phosphotungstic acid are not all known; but uric acid and purine bases, ammonia, creatinine, pigments, diamino-acids, diamines and ptomaines (if they occur), sulphocyanides, carbamic acid, urine mucoid, and proteid belong to this group. Special methods have been suggested for the determination of several of these substances (see below).

The urea nitrogen is always the greatest part of the total nitrogen, but otherwise the division of the nitrogen undergoes considerable variation. According to v. JACKSCH [2] normal human urine contains from 1.5 to 3 per cent of the total nitrogen as amino-acid nitrogen and 5.16 to 8.5 per cent as ammonia and purine bodies. Other experimenters have obtained different results, and our knowledge on this subject is not sufficient. Very great variations seem to occur not only in the healthy individual, but also and to a greater degree in diseased conditions.[3]

Formation of Urea in the Organism. The experiments to produce urea directly from proteins by oxidation have led to the formation of some guanidine, but urea has not been obtained positively. On the hydrolysis of proteins arginine has been found among other products, and as it is also produced in tryptic digestion, it is possible that a small portion of the urea is produced in this manner, varying according to the kind of protein (DRECHSEL, KOSSEL, see Chapter III). DRECHSEL claims that about 10 per cent of the urea can be accounted for in this way.

The possibility of a formation of urea from arginine has gained in

[1] Zeitschr. f. physiol. Chem., **30**.

[2] Zeitschr. f. klin. Med., **50**.

[3] See Satta, Hofmeister's Beiträge, **6**, which also gives the literature, and Erben, Zeitschr. f. Heilkunde, **25**.

interest since KOSSEL and DAKIN have discovered the presence of an enzyme, *arginase*, in the liver and other organs, which has the power of splitting arginine with the formation of urea. THOMPSON [1] has recently given a direct proof for the formation of urea from arginine. The introduction of arginine into the body of a dog either per os or subcutaneously has in his experiments led to an elimination of urea. While outside of the body only one-half of the nitrogen of arginine is split off as urea and the other half as ornithine, in the above experiments the increase in urea in several instances corresponded to the greater part if not the whole of the nitrogen of the arginine introduced. In these cases, without mentioning that the arginine seemed to raise the nitrogen catabolism, probably also urea was formed from the ornithine. This can be explained by a deamidation of the ornithine and formation of urea from the ammonia and carbon dioxide split off.

By the action of alkalies, as above mentioned (Chapter XI), urea may be formed from creatinine; still such an orgin of urea in the animal body has not thus far been proven.

The amino-acids are considered as special mother-substances of urea. By the researches of SCHULTZEN and NENCKI and SALKOWSKI with leucine and glycocoll, those of STOLTE with several amino-acids, and those of v. KNIERIEM with asparagin, it has been shown that the amino-acids are in part converted into urea in the animal organism. The investigations by SALASKIN with the three amino-acids, glycocoll, leucine, and aspartic acid, have unmistakably shown that the surviving dog-liver, supplied with arterial blood, has the property of transforming the above amino-acids into urea or a closely allied substance. The researches of LOEWI with the "urea-forming" enzyme of the liver, discovered by RICHET, on glycocoll or leucine, as also the researches of ASCOLLI,[2] have led to similar results, but it must be remarked that we have no proof as to the identity of the newly formed substance with urea. The formation of urea from amino-acids is considered as proven, and, like the amino-acids, the polypeptides are also decomposed into urea in the animal organism, as shown by ABDERHALDEN with TERUUCHI and BABKIN and with SCHITTENHELM.[3]

Nothing positive can be said in regard to the manner in which this

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[1] Kossel and Dakin, Zeitschr. f. physiol. Chem., **41**; Thompson, Journ. of Physiol., **32** and **33**.

[2] Schultzen and Nencki, Zeitschr. f. Biologie, **8**; v. Knieriem, *ibid.*, **10**; Salkowski, Zeitschr. f. physiol. Chem., **4**; Salaskin, *ibid.*, **25**; Loewi, *ibid.*, **25**; Stolte, Hofmeister's Beiträge, **5**; Richet, Compt. rend., **118**, and Compt. rend. Soc. biol., **49**; Ascoli, Pflüger's Arch., **72**.

[3] Abderhalden with Teruuchi and with Babkin, Zeitschr. f. physiol. Chem., **47**, with Schittenhelm, *ibid.*, **51**.

formation of urea occurs; but there is no doubt that a formation of ammonia is here of great importance.

The possibility of a formation of urea from ammonia has been positively shown. Thus the researches of v. KNIERIEM, SALKOWSKI, FEDER, I. MUNK, CORANDA, SCHMIEDEBERG and FR. WALTER, HALLERVORDEN, and POHL and MÜNZER,[1] on the behavior of ammonium salts in the animal body and the elimination of the ammonia under various conditions, have shown that not only ammonium carbonate, but also those ammonium salts which are burnt into carbonate in the organism, are transformed into urea by carnivora as well as herbivora. v. SCHROEDER,[2] by irrigating the surviving dog's liver with blood treated with ammonium carbonate or ammonium formate, has shown that the formation of urea takes place, at least in part, in this organ. NENCKI, PAWLOW, ZALESKI and SALASKIN[3] have also found that, in dogs, the quantity of ammonia in the blood from the portal vein is considerably greater than that from the hepatic vein, and they claim that the liver retains in great part the ammonia thus supplied. The formation of urea from ammonia in the liver is a positively proven fact, and the urea formation from ammonium carbonate is to be considered as a synthesis with the elimination of water.

The assumption of a splitting off of ammonia from amino-acids is not difficult of conception, as now, especially from the investigations mentioned in Chapter VIII, we know with positiveness that deamidation of amino-acids does take place in the animal body. The ammonia split off finds in the blood and tissues the carbon dioxide necessary for the formation of carbonate, and the investigations of NOLF, as well as those of MACLEOD and HASKINS,[4] on the equilibrium of carbonate and carbamate solutions and the conditions for the formation of both salts, must also be abundant evidence of a carbamate formation.

Important observations have been made which give support to the views of SCHULTZEN and NENCKI,[5] namely, that the amino-acids are transformed into urea with carbamic acid as an intermediate step. DRECHSEL has shown that the amino-acids yield carbamic acid by oxidation in alkaline fluid outside of the organism, and he obtained urea from ammonium carbamate by passing an alternating electric current through its solution, i.e., by alternate oxidation and reduction. DRECHSEL has

[1] v. Knieriem, Zeitschr. f. Biologie, **10**; Feder, *ibid.*, **13**; Salkowski, Zeitschr. f. Biologie, **1**; Munk, *ibid.*, **2**; Coranda, Arch. f. exp. Path. u. Pharm., **12**; Schmiedeberg and Walter, *ibid.*, **7**; Hallervorden, *ibid.*, **10**; Pohl and Münzer, Arch., f. exp. Path. u. Pharm., **43**.

[2] Arch. f. exp. Path. u. Pharm., **15**. See also Solomon, Virchow's Arch., **97**.

[3] Arch. des sciencs biol. de St. Pétersbourg, **4**; see also Chapter VI, p. 317.

[4] Nolf, Zeitschr. f. physiol. Chem., **23**; Macleod and Haskins, Journ. of biol. Chem., **1**.

[5] Zeitschr. f. Biologie, **8**.

also been able to detect small quantities of carbamates in blood, and later in conjunction with ABEL he detected carbamic acid in alkaline horse's urine. DRECHSEL therefore accepts the theory of the formation of urea from ammonium carbamate, and believes that the alternating oxidation and reduction take place in the following way:

$$\underset{\text{Ammonium carbamate}}{H_4N.O.CO.NH_2} + O = H_2N.O.CO.NH_2 + H_2O$$

$$H_2N.O.CO.NH_2 + H_2 = \underset{\text{Urea}}{H_2N.CO.NH_2} + H_2O.$$

ABEL and MUIRHEAD [1] have later observed an abundant elimination of carbamic acid in human and dog's urine after the administration of large quantities of milk of lime, and the probability of the regular appearance of this acid in normal acid-reacting human and dog's urine has been demonstrated by M. NENCKI and HAHN [2]. These last-mentioned investigators have also given very important support to the theory of the formation of urea from ammonium carbamate by observations on dogs with ECK's fistula. NENCKI and HAHN observed violent symptoms of poisoning, in dogs fed on meat and operated upon by PAWLOW and MASSEN, and these symptoms were quite identical with those obtained on introducing carbamate into the blood. These symptoms also appear after the introduction of carbamate into the stomach, while the introduction of carbamate into the stomach of a normal dog had no action. As these observers also found that the urine of the dog on which the operation was made was richer in carbamate than that of the normal dog, they concluded that the symptoms were due to the non-transformation of the ammonium carbamate into urea in the liver, and they consider the ammonium carbamate as the substance from which the urea is derived in the mammalian liver.

The experience of ROTHBERGER and WINTERBERG was that the phenomena with meat feeding and with carbamic acid intoxication are not the same, and HAWK [3] has also arrived at other results. He observed violent toxic symptoms on abandoning meat feeding with such fistular dogs, but not always. In the latter cases the symptoms appeared on the simultaneous feeding of LIEBIG'S extract of beef, while this was without effect with a meat-free diet. The administration of sodium carbamate or intravenous injection of this salt in dogs with

[1] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1875. See also Journ. f. prakt. Chem. (N. F.), **12**, **16**, and **22**; Abel, Arch. f. (Anat. u.) Physiol., 1891; Abel and Muirhead, Arch. f. exp. Path. u. Pharm., **31**.

[2] Hahn, Massen, Nencki et Pawlow, La fistule d'Eck de la veine cave inférieure et de la veine porte, etc. Arch. des sciences biol. de St. Pétersbourg, **1**, No. 4, 1892.

[3] Rothberger and Winterberg, Zeitschr. f. exp. Path. u. Therap., **1**; Hawk, Amer. Journ. of Physiol., **21**.

Eck-fistula did not, on the contrary, produce the same toxic symptoms as occurred where meat and extract were fed, and these observations on these fistular animals do not allow of any positive conclusions as to the relation of the carbamates to the formation of urea. On the other hand neither do they contradict the assumption of such a mode of formation of the urea.

Besides the above view of the formation of urea from ammonium carbonate and carbamate, which has been called the anhydride theory, we also have the oxidation theory of Hofmeister.

F. Hofmeister[1] found in the oxidation of different members of the fatty series, as well as in amino-acids and proteins, that urea was formed in the presence of ammonia, and he therefore suggests the possibility that urea may be formed by an oxidation-synthesis. According to him, in the oxidation of nitrogenous substances a radical $CONH_2$, containing the amide group, unites at the moment of formation with the radical NH_2 remaining on the oxidation of ammonia, forming urea.

Besides the above-mentioned theories as to the formation of urea, there are others which will not be given, because the only theory which has thus far been positively demonstrated is the formation of urea in the liver from ammonium compounds and amino-acids.

The liver is the only organ in which, up to the present time, a formation of urea has been directly detected;[2] and the question arises, what importance has this urea formation which takes place in the liver? Is the urea wholly or chiefly formed in the liver?

If the liver is the only organ capable of forming urea, it is to be expected, on the extirpation or atrophy of that organ, that a reduced or, in short experiments, at least a strongly diminished elimination of urea should occur. As at least a part of the urea is formed in the liver from ammonium compounds, a simultaneous increase in the elimination of ammonia is to be expected.

The extirpation and atrophy experiments made on animals by different methods by Nencki and Hahn, Slosse, Lieblein, Nencki and Pawlow, Salaskin and Zaleski[3] have shown that sometimes a rather marked increase of ammonia and a diminished elimination of urea takes place after the operation, but that there are also cases in

[1] Arch. f. exp. Path. u. Pharm., **37**.

[2] In regard to the investigations of Prevost and Dumas, Meissner, Voit, Gréhant, Gscheidlen and Salkowski, and others, on the rôle of the kidneys in the formation of urea, see v. Schroeder, Arch. f. exp. Path. u. Pharm., **15** and **19**, and Voit, Zeitschr. f. Biologie, **4**.

[3] Nencki and Hahn, l. c.; Slosse, Arch. f. (Anat. u.) Physiol., 1890; Lieblein, Arch. f. exp. Path. u. Pharm., **33**; Nencki and Pawlow, Arch. des scienc. biol. de St. Pétersbourg, **5**. See also v. Meister, Maly's Jahresber., **25**; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **29**.

which, irrespective of the pronounced atrophy, an abundant formation of urea occurs, and no appreciable, if any, change in the proportion of ammonia to the total nitrogen and urea is observed. After shutting out from the circulation the organs of the posterior part of the body, especially the liver and kidneys, KAUFMANN [1] also found an important increase in the urea of the blood, and these different observations show that the liver is not the only organ, in the various animals experimented upon, in which urea is formed.

The observations made by numerous investigators [2] on human beings with cirrhosis of the liver, acute yellow atrophy of the liver, and phosphorus poisoning have led to the same result. These investigations teach that in certain cases the proportion of the nitrogenous substances may be so changed that urea is only 50–60 per cent of the total nitrogen, while in other cases, on the contrary, even in very extensive atrophy of the liver-cells, the formation of urea is not diminished, neither is the proportion between the total nitrogen, urea, and ammonia essentially changed. Even in the cases in which the formation of urea was relatively diminished and the elimination of ammonia considerably increased, further investigation must be instituted before it will be possible to assume a reduced ability of the organism to produce urea. An increased elimination of ammonia may, as shown by MÜNZER in the case of acute phosphorus poisoning, be dependent upon the formation of abnormally large quantitites of acids, caused by abnormal metabolism, and these acids require a greater quantity of ammonia for their neutralization according to the law of elimination of ammonia, which will be given later. That an abnormal formation of acid occurs after the cutting out of the liver has been especially shown by SALASKIN and ZALESKI.[3]

For the present we are not justified in the statement that the liver is the only organ in which urea is formed, and only continued investigation can yield further information as to the extent and importance of the formation of urea, from ammonium compounds, in the liver.

Properties and Reactions of Urea. Urea crystallizes in needles or in long, colorless, four-sided, often hollow, anhydrous rhombic prisms. It has a neutral reaction, and produces a cooling sensation on the tongue like saltpeter. It melts at 132° C. At ordinary temperatures it dissolves

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[1] Compt. rend. soc. biol., **46**, and Arch. de Physiol., (5), **6**.

[2] See Hallervorden, Arch. f. exp. Path. u. Pharm., **12**; Weintraud, *ibid.*, **31**; Münzer and Winterberg, *ibid.*, **33**; Stadelmann, Deutsch. Arch. f. klin. Med., **33**; Fawitzki, *ibid.*, **45**; Münzer, *ibid.*, **52**; Fränkel, Berlin. klin. Wochenschr., 1878; Richter, *ibid.*, 1896; Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**, and Sjöqvist, Nord. Med. Arkiv, 1892; Gumlich, Zeitschr. f. physiol. Chem., **17**; v. Noorden, Lehrb. d. Pathol. des Stoffwechsels, 2. Aufl., Bd. 1, 104.

[3] Zeitschr. f. physiol. Chem., **29**.

in an equal weight of water and in five parts alcohol; it requires one part boiling alcohol for solution; it is insoluble in alcohol-free anhydrous ether, and also in chloroform. If urea in substance is heated in a test-tube, it melts, decomposes, gives off ammonia, and finally leaves a non-transparent white residue which, among other substances, contains cyanuric acid and *biuret*, which latter dissolves in water, giving a beautiful reddish-violet liquid with copper sulphate and alkali (*biuret reaction*). On heating with baryta-water or caustic alkali, also in the so-called alkaline fermentation of urine caused by micro-organisms, urea splits into carbon dioxide and ammonia with the addition of water. The same decomposition products are produced when urea is heated with concentrated sulphuric acid. An alkaline solution of sodium hypobromite decomposes urea into nitrogen, carbon dioxide, and water according to the equation

$$CON_2H_4+3NaOBr=3NaBr+CO_2+2H_2O+N_2.$$

With a concentrated solution of furfurol and hydrochloric acid urea in substance gives a coloration passing from yellow, green, blue, to violet, and then after a few minutes beautiful purple-violet (SCHIFF'S reaction). According to HUPPERT [1] the test is best performed by taking 2 cc. of a concentrated furfurol solution, 4–6 drops of concentrated hydrochloric acid, and adding to this mixture, which must not be red, a small crystal of urea. A deep violet coloration appears in a few minutes.

Urea forms crystalline compounds with many acids. Among these the one with nitric acid and the one with oxalic acid are the most important.

UREA NITRATE, $CO(NH_2)_2.HNO_3$. On crystallizing quickly this compound forms thin rhombic or six-sided overlapping tiles, or colorless plates, with an angle of 82°. When crystallizing slowly, larger and thicker rhombic pillars or plates are obtained. This compound is rather easily soluble in pure water, but is considerably less soluble in water containing nitric acid; it may be obtained by treating a concentrated solution of urea with an excess of strong nitric acid free from nitrous acid. On heating this compound it volatilizes without leaving a residue.

This compound may be employed with advantage in detecting small amounts of urea. A drop of the concentrated solution is placed on a microscope slide and the cover-glass placed upon it; a drop of nitric acid is then placed on the side of the cover-glass and allowed to flow under. The formation of crystals begins where the solution and the nitric acid meet. Alkali nitrates may crystallize very similarly to urea nitrate when they are contaminated with other bodies; therefore, in testing for urea, the crystals must be identified as urea nitrate by heating and by other means.

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[1] Huppert-Neubauer, Analyse des Harns, 10. Aufl., 296.

Urea Oxalate, $2.CO(NH_2)_2.H_2C_2O_4$. This compound is more sparingly soluble in water than the nitric-acid compound. It is obtained in rhombic or six-sided prisms or plates on adding a saturated oxalic-acid solution to a concentrated solution of urea.

Urea also forms combinations with mercuric nitrate in variable proportions. If a very faintly acid mercuric-nitrate solution is added to a 2 per cent solution of urea and the mixture carefully neutralized, a compound is obtained of a constant composition which contains for every 10 parts of urea 72 parts of mercuric oxide. This compound serves as the basis of Liebig's titration method. Urea also combines with salts, forming mostly crystallizable combinations, as, for instance, with sodium chloride, with the chlorides of the heavy metals, etc. An alkaline but not a neutral solution of urea is precipitated by mercuric chloride.

If urea is dissolved in dilute hydrochloric acid and then an excess of formaldehyde is added, a thick, white, granular precipitate is obtained which is difficultly soluble and whose composition is somewhat disputed.[1] With phenylhydrazine, urea in strong acetic acid gives a colorless crystalline compound of phenylsemicarbazid, $C_6H_6NH.NH.CONH_2$, which is soluble with difficulty in cold water and melts at 172° C. (Jaffé [2]).

The method of preparing urea from urine is in the main as follows: Concentrate the urine, which has been faintly acidified with sulphuric acid, at a low temperature, add an excess of nitric acid, at the same time keeping the mixture cool, press the precipitate well, decompose it in water with freshly precipitated barium carbonate, dry on the water-bath, extract the residue with strong alcohol, decolorize when necessary with animal charcoal, and filter while warm. The urea which crystallizes on cooling is purified by recrystallization from warm alcohol. A further quantity of urea may be obtained from the mother-liquor by concentration. The urea is purified from contaminating mineral bodies by redissolving in alcohol-ether. If it is only necessary to detect the presence of urea in urine, it is sufficient to concentrate a little of the urine on a watch-glass and, after cooling, treat it with an excess of nitric acid. In this way we obtain crystals of urea nitrate.

Quantitative Estimation of the Total Nitrogen and Urea in Urine. Among the various methods proposed for the estimation of the total nitrogen, that suggested by Kjeldahl is to be recommended. Liebig's method for the estimation of urea is really a method for determining the total nitrogen, but as it is very seldom used now we can refer to larger works in regard to details.

Kjeldahl's method consists in transforming all the nitrogen of the organic substances into ammonia by heating with a sufficiently concentrated sulphuric acid. The ammonia is distilled off after super-

[1] See Tollens and his pupils, Ber. d. deutsch. chem. Gesellsch., **29**, 2751; Goldschmidt, *ibid.*, **29**, and Chem. Centralbl., 1897, **1**, 33; Thoms, *ibid.*, **2**, 144 and 737.

[2] Zeitschr. f. physiol. Chem., **22**.

saturating with alkali and the ammonia collected in standard sulphuric acid. The following reagents are necessary:

1. *Sulphuric Acid.* Either a mixture of equal volumes of pure concentrated and fuming sulphuric acid or else a solution of 200 grams phosphoric anhydride in 1 liter of pure concentrated sulphuric acid. 2. *Caustic soda* free from nitrates, 30–40 per cent solution. The quantity of this caustic-soda solution necessary to neutralize 10 cc. of the acid mixture must be determined. 3. Metallic *mercury* or pure yellow *mercuric oxide.* (The addition of this facilitates the destruction of the organic substances.) 4. A *potassium-sulphide* solution of 4 per cent, whose object is to decompose any mercuric amide combination which might not evolve its ammonia completely during the distillation with caustic soda. 5. 1/5 normal sulphuric acid and 1/5 normal caustic soda solution.

In performing the determination 5 cc. of the carefully measured and filtered urine are placed in a long-necked KJELDAHL flask, a drop of mercury or about 0.3 gram of mercuric oxide added, and then treated with 10–15 cc. of the strong sulphuric acid. The contents are heated very carefully, placing the flask at an angle, until they just begin to boil gently; this is continued for about half an hour after the mixture becomes colorless. On cooling the contents are transferred to a voluminous distilling-flask, carefully washing the KJELDAHL flask with water, and the greater part of the acid is neutralized by caustic soda. A few zinc shavings are added to prevent too rapid ebullition on distillation, and then an excess of caustic-soda solution which has previously been treated with 30–40 cc. of the potassium-sulphide solution. The flask is quickly connected with the condenser-tube and all the ammonia distilled off. In order to prevent loss of ammonia it is best to lower the end of the exit-tube below the surface of the acid, the regurgitation of the acid being prevented by having a bulb blown on the exit-tube. Not less than 25–30 cc. of the standard acid is used for every 5 cc. of urine, and on completion of the distillation the acid is retitrated with 1/5 normal caustic soda, using rosolic acid, tincture of cochineal, or lacmoid as indicator. Each cubic centimeter of the acid corresponds to 2.8 milligrams nitrogen. As a control and in order to test the purity of the reagents, or to eliminate any error caused by an accidental quantity of ammonia in the air, we always make a blank determination with the reagents.

Among the methods suggested for the special estimation of urea, that of MÖRNER-SJÖQVIST, in combination with FOLIN'S method, is perhaps the most trustworthy and readily performed. For this reason only this method will be given in detail, while we must refer to special works for the other methods, such as BUNSEN'S method with its many modifications as suggested by PFLÜGER, BOHLAND and BLEIBTREU.[1]

Principle of Mörner-Sjöqvist's Method.[2] According to this method the nitrogenous constituents of the urine, with the exception of urea,

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[1] Pflüger's Arch., **38**, **43**, and **44**.

[2] Skand. Arch. f. Physiol., **2**, and Mörner, *ibid.*, **14**, where the recent literature may also be found.

ammonia, hippuric acid, creatinine, and traces of allantoin,[1] are precipitated by a mixture of alcohol and ether after the addition of a solution of barium chloride and barium hydroxide or in the presence of sugar with solid barium hydroxide. The urea is determined in the concentrated filtrate, after driving off the ammonia, by KJEDLDAHL'S nitrogen estimation. Because of the slight error due to the presence of hippuric acid and creatinine, several modifications have been suggested by SALASKIN and ZALESKI and by BRAUNSTEIN.[2] These errors are best prevented, according to MÖRNER, by the use of FOLIN'S method.

Principle of Folin's Method.[3] On heating urea with hydrochloric acid and crystalline magnesium chloride, which melts in its water of crystallization at 112–115° C. and then boils at about 150–155° C., the urea is completely decomposed, while no appreciable decomposition of the hippuric acid and creatinine takes place. The ammonia produced from the urea is distilled off and determined by titration. The amount of ammonia previously existing in the urine must be specially determined.

Determination of Urea by the Mörner–Sjöqvist and Folin Method.[4] Five cc. of the urine are treated with 1.5 grams of powdered barium hydroxide, and when as much of this is dissolved as possible by gently mixing, it is precipitated by 100 cc. of the alcohol and ether mixture (¼ vol. ether). On the following day it is filtered and the precipitate washed with the alcohol and ether mixture. The alcohol and ether are distilled off from the filtrate at about 55° C. (not above 60° C.). The remaining liquid is treated with 2 cc. of hydrochloric acid of sp.gr. 1.124 (for 5 cc. urine), and carefully transferred to a flask of 200 cc. capacity, and evaporated to dryness on the water-bath. Then add 20 grams of crytalline magnesium chloride to the contents of the flask and 2 cc. of concentrated hydrochloric acid, and boil on a wire gauze over a small flame for two hours, making use of a proper return cooler. After cooling it is diluted to about ¾ to 1 liter with water, the ammonia completely distilled off after making it alkaline with caustic soda, and the ammonia collected in standard acid. After boiling in order to drive off the CO_2 and cooling, the acid is retitrated. Corrections must be made for the ammonia of the urine and for that contained in the magnesium chloride.

If a special determination of the preformed ammonia has been made, then a direct treatment of the urine, according to FOLIN (nevertheless after the evaporation of the urine with hydrochloric acid), gives good results. In the presence of sugar the treatment of the urine with barium hydroxide is absolutely necessary in MÖRNER'S opinion, otherwise the humin substances produced from the sugar take up and retain nitrogen.

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[1] According to Wiechowski, Hofmeister's Beiträge, **11**, the quantity of allantoin is so great in urine that it must be considered in this method.

[2] Braunstein, Zeitschr. f. physiol. Chem., **31**; Salaskin and Zaleski, *ibid.*, **28**.

[3] *Ibid.*, **32**, **36**, and **37**.

[4] See Mörner, Skand. Arch. f. Physiol., **14**.

HASKINS has changed FOLIN'S method, as he precipitates the urine first with phosphomolybdic acid and after a further preparation proceeds according to FOLIN. According to GLAESSNER[1] the MÖRNER-SJÖQVIST method cannot be used in the presence of large quantities of amino-acids, as they remain in part in the alcohol-ether solution.

KNOP-HÜFNER'S method[2] is based on the fact that urea, by the action of sodium hypobromite, splits into water, carbon dioxide (which dissolves in the alkali), and nitrogen, whose volume is measured (see page 653). This method is less accurate than the preceding ones, and therefore in scientific work it is discarded. It is of value to the physician and for practical purposes, because of the ease and rapidity with which it may be performed, even though it may not give very accurate results. For practical purposes a number of different apparatus have been constructed to facilitate the use of this method.

For the quantitative estimation of urea in blood or other animal fluids, as well as in the tissues, SCHÖNDORFF has proposed a method where the proteins and extractives are first precipitated by a mixture of phosphotungstic acid and hydrochloric acid, and then the filtrate made alkaline with lime. The quantity of ammonia formed on heating a part of this filtrate to 150° C. with phosphoric acid and the amount of carbon dioxide produced by heating the other part to 150° C. are determined. In regard to the principles of this method, as well as to the details, we refer to the original article (PFLÜGER'S Arch., 62). See also HOPPE-SEYLER-THIERFELDER'S Handbuch, 8. Aufl. SALKOWSKI[3] has recently suggested a method for estimating the urea in tissues.

Urein is the name given by OVID MOOR to a product which he obtained by extracting urine, which had been evaporated to a syrup, with absolute alcohol and precipitating the urea with alcohol containing oxalic acid, or by cooling and treatment with alcohol. Urein is a golden-yellow oil which is poisonous; it reduces permanganate in the cold, and it forms the chief portion of the nitrogenous extractives of urine. There is no doubt that urein is a mixture of several substances. According to MOOR,[4] the amount of urea in the urine is only about one-half that ordinarily given, and he has suggested a new method for the determination of the true quantity of urea. The possibility that in the urine we have other bodies besides urea which have been determined with the urea cannot be denied *a priori*. From the investigations published so far it must be said that MOOR'S assertions are not sufficiently grounded.[5]

Carbamic Acid, $CH_3NO_2=CO\begin{<}NH_2\\OH\end{>}$. This acid is not known in the free state, but only as salts. Ammonium carbamate is produced by the action of dry ammonia on dry carbon dioxide, but also after the addition of Na_2CO_3 to a

[1] Haskins, Journ. of biol. Chem., **2**; Glaessner, Zeitschr. f. exp. Path. u. Therap., **4**.

[2] Knop, Zeitschr. f. analyt. Chem., **9**; Hüfner, Journ. f. prakt. Chem. (N. F.), **3**. In regard to the extensive literature, see Huppert-Neubauer, 10. Aufl., 304, and following.

[3] Arbeiten aus dem pathol. Institute, Berlin, 1906.

[4] O. Moor, Bull. Acad. de St. Pétersbourg, **14** (also Maly's Jahresber., **31**, 415), and Zeitschr. f. Biologie, **44** and **45**, and Zeitschr. f. physiol. Chem., **40** and **48**.

[5] See Kubiabko, Maly's Jahresber., **31**, 415; Erben, Zeitschr. f. physiol. Chem. **38**; Folin, *ibid.*, **37**; Gies, Journ. Amer. Chem. Soc., **25**; Haskins, Amer. Journ. of Physiol., **12**; Lippich, Zeitschr. f. physiol. Chem., **48** and **52**.

solution which contains an ammonium salt (MACLEOD and HASKINS). Carbamic acid is also produced by the action of potassium permanganate on protein and several other nitrogenous organic bodies.

The occurrence of carbamic acid in human and animal urines has already been considered in connection with the formation of urea. The calcium salt, which is soluble in water and ammonia but insoluble in alcohol, is the most important in the detection of this acid. The solution of the calcium salt in water becomes cloudy on standing, but much more quickly on boiling, and calcium carbonate separates. NOLF, MACLEOD and HASKINS have made experiments as to the method of formation of carbamic acid. The latter have indicated a new method for the quantitative estimation of carbamates.[1]

Carbamic-acid ethylester (urethane), as shown by JAFFÉ,[2] may pass, by the mutual action of alcohol and urea, into the alcoholic extract of urine when one is working with large quantities.

FOLIN [3] claims that all human urine contains a body which is probably *methylurea*.

Creatinine, $C_4H_7N_3O$, or $NH:C\left\langle\begin{array}{l}NH\text{——}CO\\ \qquad\quad\ |\\ N(CH_3).CH_2\end{array}\right.$, is generally considered as the anhydride of creatine (see page 546) found in the muscles. It occurs in human urine and in that of certain mammalia. It has also been found in ox-blood, milk, though in very small amounts, and in the flesh of certain fishes.

JOHNSON's statement that the creatinine of the urine is different from that produced by the action of acids on creatine is incorrect according to TOPPELIUS and POMMEREHNE, WOERNER and THELEN.[4]

The quantity of creatinine in human urine is, in a grown man voiding a normal quantity of urine in the course of a day, 0.6–1.3 grams (NEUBAUER), or on an average 1 gram. JOHNSON [5] found 1.7–2.1 grams per day, and similar results have been obtained by v. HOOGENHUYZE and VERPLOEGH.[6] The quantity of creatinine with a diet free from meat is, FOLIN [7] says, variable for different individuals, but is constant for the same person. He never found the quantity below 1 gram and often between 1.3 and 1.7 grams. Nurslings also eliminate creatinine, although the quantity is small (v. HOOGENHUYZE and VERPLOEGH). The quantity of creatinine nitrogen in per cent of the total nitrogen varies under different conditions, but is on an average about 4.5–6 per cent, as determined by several experimenters.

As the two bodies, creatine and creatinine, can easily be transformed

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[1] Nolf, Zeitschr. f. physiol. Chem., **23**; Macleod and Haskins, Amer. Journ. of Physiol., **12**, and Journ. of biol. Chem., **1**.

[2] Zeitschr. f. physiol. Chem., **14**.

[3] Journ. [illegible].

[4] According to Wiech. Roy. Soc., **42**, **43**; Chem. News, **55**; Toppelius and Pommerehne, in urine therm., **234**; Woerner, Arch. f. (Anat. u.) Physiol., 1898.

[5] Huppertein, Zeauer, Harnanalyse, 10. Aufl., 387.

[6] Zeitschr. f. physiol. Chem., **46**.

[7] Amer. Journ. of Physiol. **13**; v. Klercker, Hofmeister's Beiträge, 8.

into each other, it has been considered for a long time that the urinary creatinine is formed from the creatine of the muscles and other organs. Unfortunately the authorities disagree on this question. FOLIN in his investigations found that about 80 per cent of the creatinine introduced was again eliminated, while the creatine taken did not appear in the urine as creatinine, but was partly retained by the body and in part eliminated as such. An intravital transformation of creatine into creatinine is disputed by v. KLERCKER, MELLANBY and LEFMANN,[1] while it is accepted by GOTTLIEB, STANGASSINGER, S. WEBER, v. HOOGENHUYZE and VERPLOEGH and ROTHMANN, partly based upon autolytic experiments (see Chapter XI). According to v. HOOGENHUYZE and VERPLOEGH[2] a part of the creatine formed in the body is oxidized and a part changed into creatinine.

The proteins, or rather the guanidine groups therein, are considered as the probable mother substance of these two bodies. JAFFÉ has indeed shown, which has been substantiated later by DORNER,[3] that in rabbits glycocyamine (guanidine acetic acid) is in part changed into creatine with the annexation of methyl. Guanidine occurs in the proteins as arginine, but the observations of OTORI[4] show that it is not improbable that the proteins also contain other guanidine groups. JAFFÉ considers it improbable that the arginine is the mother substance of the creatine, but rather another guanidine group. Under the circumstances the proteins can be considered as the mother substance of the creatine and creatinine, and in a previous chapter (XI) the experiments of SEEMAN[5] showed the abundant formation of creatine from protein by autolysis.

If then the creatinine (creatine) originates from the protein, it is evident that we must differentiate between food protein and body protein. The quantity of creatinine is, inasmuch as it is increased by meat diet, dependent upon the food; but otherwise, as found by FOLIN and in chief substantiated by others,[6] is rather independent of the food. Its elimination does not run parallel with the urea and the total nitrogen, and consequently is not in general greater with food rich in protein than with food poor therein. On the contrary, its extent, as shown by other conditions, is dependent upon the intensity of the metabolism in the cells, and the creatinine, according to FOLIN, is a product of the endogenous protein metabolism.

[1] Folin, Hammarsten's Festschrift, 1906; v. Klercker, Bioch. Zeitschr., **3**; Mellanby, Journ. of Physiol., **36**; Lefmann, Zeitschr. f. physiol. Chem., **57**.

[2] See footnote 1, page 547, and v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., **59**.

[3] Jaffé, Zeitschr. f. physiol. Chem., 48; Dorner, *ibid.*, **52**.

[4] Zeitschr. f. physiol. Chem., **42**, **43**.

[5] l. c., footnote 1, page 547.

[6] Besides the works cited above see also Closson, Amer. Journ. of Physiol., **16**.

Reports as to the behavior of the creatinine elimination with work are conflicting. v. HOOGENHUYZE and VERPLOEGH, who made use of a much more trustworthy method of quantitative estimation than their predecessors, find that muscular activity as a rule does not cause any rise in the creatinine elimination, and that in man such a rise with work occurs only when the body is obliged to live upon its own tissues. S. WEBER[1] also finds an absolute increase in the elimination of creatinine only in starving dogs.

In starvation a decrease in the creatinine but a simultaneous increase in the elimination of creatine has been found in man (v. HOOGENHUYZE and VERPLOEGH, CATHCART, BENEDICT and MYERS[2]). Little is known about the behavior of creatinine in disease nor are the observations in accord. In anæmia and cachexia the elimination of creatinine is diminished, and when the metabolism is increased the elimination is also increased. That this is the case, at least in fevers, seems to be borne out by several concurrent observations.[3] In diseases of the liver a diminished elimination of creatinine may occur and in cases of carcinoma of the liver considerable creatine has been found in the urine (v. HOOGENHUYZE and VERPLOEGH, MELLANBY).

Creatinine crystallizes in colorless, shining monoclinic prisms which differ from creatine crystals in not becoming white with loss of water when heated to 100° C. It dissolves in 11 parts cold water, but more easily in warm water. It is difficultly soluble in cold alcohol, but the reports in regard to its solubility differ widely.[4] It is more soluble in warm alcohol and nearly insoluble in ether. In alkaline solution creatinine is very easily converted into creatine on warming.

Creatinine gives an easily soluble crystalline compound with hydrochloric acid. A solution of creatinine acidified with mineral acids gives crystalline precipitates with phosphotungstic and phosphomolybdic acids even in very dilute solutions (1:10000) (KERNER, HOFMEISTER[5]). It is precipitated, like urea, by mercuric-nitrate solution and also by mercuric chloride. On treating a dilute creatinine solution with sodium acetate and then with mercuric chloride a precipitate of glassy globules having the composition $4(C_4H_7N_3O.HCl.HgO)3HgCl_2$ separates on standing some time (JOHNSON). Among the compounds of creatinine, that

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[1] Arch. f. exp. Path. u. Pharm., 58. Further literature may be found in v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., 46.

[2] v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., 57; Cathcart, Bioch. Zeitschr., 6; Benedict and Myers, Amer. Journ. of Physiol., 18; Jaffé, l. c.

[3] See O. v. Klercker, Zeitschr. f. klin. Med., 68.

[4] See Huppert-Neubauer, 10. Aufl., and Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

[5] Kerner, Pflüger's Arch., 2; Hofmeister, Zeitschr. f. physiol. Chem., 5.

with zinc chloride, *creatinine-zinc chloride*, $(C_4H_7N_3O)_2ZnCl_2$, is of special interest. This combination is obtained when a sufficiently concentrated solution of creatinine in alcohol is treated with a concentrated, faintly acid solution of zinc chloride. Free mineral acids dissolve the compound, hence they must not be present; this, however, may be prevented by an addition of sodium acetate. In the impure state, as from urine, creatinine-zinc chloride forms a sandy, yellowish powder which under the microscope appears as fine needles, forming concentric groups, mostly complete rosettes or yellow balls or tufts, or grouped as brushes. On slowly crystallizing or when very pure, more sharply defined prismatic crystals are obtained. The compound is slightly soluble in water.

Creatinine acts as a reducing agent. Mercuric oxide is reduced to metallic mercury, and oxalic acid and methylguanidine (methyluramine) are formed. Creatinine also reduces cupric hydroxide in alkaline solution, forming a colorless soluble compound, and only after continued boiling with an excess of copper salt is free suboxide of copper formed. Creatinine interferes with TROMMER'S test for sugar, partly because it has a reducing action, and partly by retaining the copper suboxide in solution. The compound with copper suboxide is not soluble in a saturated soda solution, and if a little creatinine is dissolved in a cold saturated soda solution and then a few drops of FEHLING'S reagent added, a white flocculent compound separates after heating to 50–60° C. and then cooling (v. MASCHKE'S [1] reaction). An alkaline bismuth solution (see Sugar Tests) is not reduced by creatinine.

An aqueous solution of creatinine is precipitated by picric acid. The precipitate consists on recrystallization from hot water, of thin, silky, pale yellow needles (JAFFÉ). If the urine is treated with picric acid (20 cc. of a 5-per cent solution in alcohol for each 100 cc. urine), then a double picrate of creatinine and potassium is precipitated (JAFFÉ). If a solution of creatinine in water (or urine) is treated with a watery solution of picric acid and a few drops of a dilute caustic-soda solution, a red coloration, lasting several hours, immediately occurs at the ordinary temperature, which turns yellow on the addition of acid (JAFFÉ'S [2] reaction). Acetone gives a more reddish-yellow color. Dextrose gives with this reagent a red coloration only after heating.

If we add a few drops of a freshly prepared very dilute sodium-nitroprusside solution (sp.gr. 1.003) to a dilute creatinine solution (or to the urine) and then a few drops of caustic soda, a ruby-red liquid is obtained which quickly turns yellow again (WEYL'S [3] reaction). If the cold yellow solution is neutralized and treated with an excess of acetic acid,

[1] Zeitschr. f. analyt. Chem., **17**.

[2] Zeitschr. f. physiol. Chem., **10**.

[3] Ber. d. deutsch. chem. Gesellsch., **11**.

a crystalline precipitate of a nitroso-compound ($C_4H_6N_4O_2$) of creatinine separates on stirring (KRAMM [1]). If, on the contrary, the yellow solution is treated with an excess of acetic acid and heated, the solution becomes first green and then blue (SALKOWSKI [2]); finally a precipitate of Prussian blue is obtained.

A reaction which in description is similar and which, although not solely (ARNOLD) but at least partially (HOLOBUT), appears after partaking of protein food or meat soup is ARNOLD'S reaction.[3] This reaction is not due to creatinine. If 10–20 cc. urine are treated with a few drops of a 4 per cent sodium nitroprusside solution and then with 5–10 cc. of a 5 per cent sodium or potassium hydroxide solution, at first a strong and pure violet color is obtained with an absorption band between D and E, then it becomes purple-red and then brown-red and finally yellow. On the addition of acetic acid the violet or purple-red color passes into blue, which soon becomes pale and finally a pale yellow color. It differs from the creatinine in color and absorption band as well as in that the creatinine reaction requires more sodium nitroprusside.

In preparing creatinine from urine the creatinine-zinc chloride is first prepared according to NEUBAUER'S [4] method. Creatinine may also be prepared from urine by precipitating with a mercuric-chloride solution according to either MALY'S or JOHNSON'S [5] process.

The best method for preparing creatinine is the following, suggested by FOLIN.[6] The creatinine is first precipitated as the double picrate of creatinine and potassium by means of picric acid according to JAFFÉ'S method, and then this precipitate, while still moist, is decomposed by $KHCO_3$ and water. The solution, which contains the creatinine besides potassium carbonate and small amounts of impurities, is neutralized with sulphuric acid and the sulphate precipitated by alcohol. The creatinine is now converted into the double zinc-chloride salt and this last treated with moist lead hydroxide. After the removal of the lead, the solution contains a mixture of creatinine and creatine, which last is completely transformed into creatinine by heating for forty-eight hours with normal sulphuric acid. After exact neutralization with barium-hydroxide solution it is concentrated to the point of crystallization.

The *quantitative estimation of creatinine* used to be performed according to NEUBAUER'S method for the preparation of creatinine, or more simply by SALKOWSKI'S[7] modification of this method. As this method is now seldom used we refer the reader to other hand-books.

FOLIN [8] has suggested a colorimetric method for determining creatinine which is based upon JAFFÉ'S picric-acid reaction and is as follows: 10 cc. of the urine are treated in a graduated flask of 500 cc. capacity with 15 cc. of a 1.2 per cent solution of picric acid and 5 cc. of a 10 per cent NaOH solution. After shaking and allowing to stand for five

[1] Centralbl. f. d. med. Wissensch., 1897.

[2] Zeitschr. f. physiol. Chem., **4**.

[3] Arnold, Zeitschr. f. physiol. Chem., **49**; Holobut, *ibid.*, **56**.

[4] Ann. d. Chem. u. Pharm., **119**.

[5] Maly, Annal. d. Chem. u. Pharm., **159**; Johnson, Proceed. Roy. Soc., **43**.

[6] Zeitschr. f. physiol. Chem., **41**.

[7] *Ibid.*, **10** and **14**.

[8] *Ibid.*, **41**.

minutes it is diluted with water to 500 cc. and mixed. This solution is now compared in a DUBOSCQ colorimeter with a 1/2 normal potassium-dichromate solution. The latter solution has in a layer 8 mm. thick exactly the same intensity of color as a layer 8.1 mm. thick of a solution of 10 milligrams creatinine after the addition of 15 cc. picric-acid solution and 5 cc. NaOH solution and dilution to 500 cc. The calculations are simple. For example, in case the urine tested in a layer 7.2 mm. thick has the same color as the dichromate solution in a layer 8 mm. thick, then the quantity of creatinine in 10 cc. of the urine will be $=\frac{8.1}{7.2}\times 10$, or 11.25 milligrams. This method has been tried by many authorities and found to be trustworthy.

The same method is used in the determination of creatine, which for this purpose is first converted into creatinine by warming with dilute mineral acid. The quantity of creatine is the difference obtained between the values for creatinine before and after treatment with acid. More detailed directions can be found in the cited works of FOLIN, v. HOOGENHUYZE and VERPLOEGH, GOTTLIEB and STANGASSINGER.

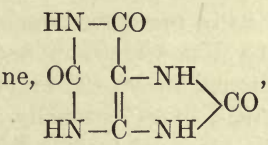
In regard to other methods, see the works of KOLISCH and GREGOR.[1]

Xanthocreatinine, $C_5H_{10}N_4O$. This body, which was first prepared from meat extract by GAUTIER, has been found by MONARI in dog's urine after the injection of creatinine into the abdominal cavity, and in human urine after several hours of exhaustive marching. According to COLASANTI it occurs to a relatively greater extent in lion's urine. STADTHAGEN [2] considers the xanthocreatinine isolated from human urine after strenuous muscular activity as impure creatinine.

Xanthocreatinine forms thin sulphur-yellow plates, similar to cholesterin, which have a bitter taste. It dissolves in cold water and in alcohol, and gives a crystalline compound with hydrochloric acid and a double compound with gold and platinum chloride. It gives a compound with zinc chloride, which crystallizes in fine needles. Xanthocreatinine has a poisonous action.

Methylguanidine occurs, according to ACHELIS, KUTSCHER and LOHMANN, to a slight extent as a regular constituent of the urine of man, horse and dog. It has been found in urines associated with *dimethylguanidine* by ENGELAND.[3]

Uric Acid, $\overline{\text{Ur}}$, $C_5H_4N_4O_3$; 2, 6, 8-trioxypurine,



, has been prepared synthetically by HORBACZEWSKI by fusing urea and glycocoll or by heating trichlorlactic-acid amide with an excess of urea. BEHREND and ROOSEN prepared it from isodialuric acid and urea; it is also readily produced from isouric acid on boiling with hydrochloric

[1] Kolisch, Centralbl. f. innere Med., 1895; Gregor, Zeitschr. f. physiol. Chem., **31**.

[2] Gautier, Bull. de l'acad. de méd. (2), **15**, and Bull. de la soc. chim. (2), **48**: Monari, Maly's Jahresber., **17**; Colasanti, Arch. ital. d. Biologie, **15**, Fasc. 3; Stadthagen, Zeitschr. f. klin. Med., **15**.

[3] Achelis, Centralbl. f. Physiol., **20**, 455, and Zeitschr. f. physiol. Chem., **50**; Kutscher and Lohmann, ibid., **49**; Engeland, *ibid.*, **57**.

acid (E. Fischer and Tüllner) and finally E. Fischer and Ach [1] have prepared uric acid from pseudouric acid by heating with oxalic acid to 145° C.

On strongly heating uric acid it decomposes with the formation of *urea, hydrocyanic acid, cyanuric acid,* and *ammonia.* On heating with concentrated hydrochloric acid in sealed tubes to 170° C. it splits into *glycocoll, carbon dioxide,* and *ammonia.* By the action of oxidizing agents splitting and oxidation take place, and either monoureides or diureides are produced. By oxidation with lead peroxide, *carbon dioxide, oxalic acid, urea,* and *allantoin,* which last is glyoxyldiureide, are produced (see below). By oxidation with nitric acid in the cold, *urea* and a monoureide, the mesoxalyl urea, or *alloxan,* are obtained, $C_5H_4N_4O_3 + O + H_2O = C_4H_2N_2O_4 + (NH_2)_2CO$. On warming with nitric acid, alloxan yields carbon dioxide and oxalyl urea, or *parabanic acid,* $C_3H_2N_2O_3$. By the addition of water the parabanic acid passes into *oxaluric acid,* $C_3H_4N_2O_4$, traces of which are found in the urine and which easily splits into oxalic acid and urea. In alkaline solution uric acid may, by taking up water and oxygen, be transformed into a new acid, uroxanic acid, $C_5H_8N_4O_6$, which may then be changed into oxonic acid, $C_4H_5N_3O_4$.[2] Uric acid may, as F. and L. Sestini as well as Gerard have shown, undergo bacterial fermentation with the formation of urea. According to Ulpiani and Cingolani,[3] uric acid is quantitatively split into urea and carbon dioxide, according to the equation

$$C_5H_4N_4O_3 + 2H_2O + 3O = 3CO_2 + 2CO(NH_2)_2.$$

Uric acid occurs most abundantly in the urine of birds and of scaly amphibians, in which animals the greater part of the nitrogen of the urine appears in this form. Uric acid frequently occurs in the urine of carnivorous mammalia, but is sometimes absent; in urine of herbivora it is habitually present, though only as traces; in human urine it occurs in greater but still small and variable amounts. Traces of uric acid are also found in several organs and tissues, as in the spleen, lungs, heart, pancreas, liver (especially in birds), and in the brain. It always occurs in the blood of birds. Traces have been found in human blood under normal conditions. Under pathological conditions it occurs to an increased extent in the blood, as in pneumonia and nephritis, but especially in leucæmia and sometimes also in arthritis. Uric acid also occurs

[1] Horbaczewski, Monatshefte f. Chem., **6** and **8**; Behrend and Roosen, Ber. d. d. chem. Gesellsch., **21**; Fischer and Tüllner, *ibid.*, **35**; Fischer and Ach, *ibid.*, **28**.

[2] See Sundwik, Zeitschr. f. physiol. Chem., **20** and **41**; also Behrend, Annal. d. Chem. u. Pharm., **333**.

[3] See Chem. Centralbl., 1903, where the other investigators are cited, and Centralbl. f. Physiol., **19**.

in large quantities in "chalk-stones," certain urinary calculi, and in guano. It has also been detected in the urine of insects and certain snails, as also in the wings (which it colors white) of certain butterflies (HOPKINS [1]).

The amount of uric acid eliminated with human urine is subject to considerable individual variation, but amounts on an average to 0.7 gram per day on a mixed diet. The ratio of uric acid to urea varies considerably with a mixed diet, but is on an average 1:50–1:70. In newborn infants and in the first days of life the elimination of uric acid is relatively increased, and the relation between uric acid and urea has been found to be 1:6.42–17.1.

We used to ascribe an increasing action upon the elimination of uric acid to protein food, but the investigations of HIRSCHFELD, ROSENFELD and ORGLER, SIVÉN, BURIAN and SHUR,[2] and many others have positively proven that a diet rich in protein does not itself increase the elimination of uric acid, but only according to the amount of nucleins or purine bodies contained therein. The common assumption that the elimination of uric acid is smaller with a vegetable diet than with an animal diet, when the quantity may be 2 grams or more per twenty-four hours, is explained by this.[3]

Still a purine-free diet is not without some influence upon the elimination of uric acid, as the quantity of uric acid eliminated with a purine-free diet is considerably greater than in starvation. HIRSCHSTEIN explains this by the formation of secretions containing purines due to the digestion, an explanation which BRUGSCH and SCHITTENHELM [4] do not accept. Work and rest do not seem to have any special influence upon the uric acid elimination, although according to the confirmed statement of SIVÉN and LEATHES [5] the elimination in the night is less than in the morning hours.

The reports in regard to the influence of other circumstances, as well as of different substances, on the elimination of uric acid are diverse. This is in part due to the fact that the earlier investigators used an inaccurate method (HEINTZ), and also that the extent of uric-acid elimination is dependent in the first place upon the individuality. Thus the investigators are not in accord in regard to the action of drinking-

[1] Philos. Trans. Roy. Soc., **186**, B, 661.

[2] See the extensive review of the literature in Wiener, "Die Harnsäure," in Ergebnisse der Physiologie, **1**, Abt. 1, 1902.

[3] J. Ranke, Beobachtungen und Versuche über die Ausscheidung der Harnsäure, etc. (München, 1858); Mares, Centralbl. f. d. med. Wissensch., 1888; Horbaczewski, Wien. Sitzungsber., **100**, Abt. 3, 1891. In regard to the action of various diets the reader is referred to the above-cited authors, and especially to A. Hermann, Arch. f. klin. Med., **43**, and Camerer, Zeitschr. f. Biologie, **33**, and Folin, Amer. Journ. of Physiol., **13**.

[4] Zeitschr. f. exp. Path. u. Therap., **4**; Hirschstein, Arch. f. exp. Path. u. Pharm., **57**.

[5] Sivén, Skand. Arch. f. Physiol., **11**; Leathes, Journ. of Physiol, **35**.

water [1] and of alkalies.[2] Certain medicines, such as quinine and atropine, diminish, while others, such as pilocarpine and, as it seems, salicylic acid,[3] increase the elimination of uric acid.

There is much diversity of opinion regarding the elimination of uric acid in disease,[4] although it is known that it is increased after an abundant destruction of nucleated cells as in pneumonia, after the crisis, and in leucæmia. In leucæmia in most cases not only is the elimination to the urea increased absolutely, but also relatively; and the relation between uric acid and urea (total nitrogen calculated as urea) may in lineal leucæmia even be 1:9, while under normal conditions, according to different investigators, it is 1:50 to 70 to 100. As to the behavior of uric acid in gout, authorities are by no means agreed. That the blood contains uric acid in gout has been repeatedly shown, and it is also found with a purine-free diet (Brugsch and Schittenhelm). According to the extensive investigations of Brugsch and Schittenhelm [5] the endogenous uric acid elimination (see below) is not higher in chronic gout, but rather lower than normally, and gout is more probably characterized by a diminished enzymotic formation of uric acid as well as by a decreased enzymotic destruction of uric acid. This last moment is the cause for the appearance of uric acid in the blood and its accumulation in certain tissues.

Formation of Uric Acid in the Organism. Since Horbaczewski first showed that uric acid could be produced by oxidation from the nuclein-rich spleen-pulp or nucleins outside of the body, he also showed that nucleins when introduced into the animal body caused an increase in the elimination of uric acid. These observations have been confirmed, and at the same time developed by the work of a great number of investigators, and we are sure that uric acid can be produced from purine bases either outside or inside the animal body, and also that food rich in nucleins (especially the thymus gland) increases the elimination of uric acid and purine bases (alloxuric bases [6]). The original view of Horbaczewski, that the nucleins do not directly cause an increased elimination of uric acid, but indirectly by causing a leucocytosis with a consequent destruction of leucocytes, has been generally discarded.

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[1] See Schöndorff, Pflüger's Arch., **46**, which contains the pertinent literature.

[2] See Clar, Centralbl. f. d. med. Wissensch., 1888; Haig, Journ. of Physiol., 8; and A. Hermann, Arch. f. klin. Med., **43**.

[3] See Bohland, cited from Maly's Jahresber., **26**; Schreiber and Zaudy, *ibid.*, **30**.

[4] In regard to the extensive literature on the elimination of uric acid in disease we must refer to special works on internal diseases.

[5] Zeitschr. f. exp. Path. u. Therap., **4**.

[6] As it is not within the scope of this book to enter into a discussion of the numerous researches on this subject, we will refer to Wiener, "Die Harnsäure," Ergebnisse der Physiol., **1**, Abt. 1, 1902.

At present it is considered that a direct formation of uric acid from the nucleins takes place by the transformation of the purine bases of the nucleins into uric acid.

The uric acid, in so far as it is produced from nuclein bases, is in part derived from the nucleins of the destroyed cells of the body and in part from the nucleins or free purine bases introduced with the food. It is therefore possible to admit with BURIAN and SCHUR [1] of a double origin for the uric acid as well as the urinary purines (all purine bodies of the urine, including the uric acid), namely, an *endogenous* and an *exogenous* origin. BURIAN and SCHUR attempted to determine the quantity of endogenous urinary purines by feeding with sufficient food, but as free as possible from purine bodies, and they found that this quantity was constant for every individual, while it was variable for different persons. The observations of SIVÉN, ROCKWOOD,[2] and many others have also led to the same results. Other investigators have arrived at different results, or they draw different deductions from their observations; still this does not change the essential fact that the uric acid originating from the nucleins is partly endogenous and partly exogenous, and that the amount of endogenous uric acid is only very slightly dependent upon the protein content of the food.

In man and other mammalia the greatest amount if not all of the uric acid originates from the nucleins or the purine bases. This formation of uric acid seems to be of an enzymotic kind. After it was shown that certain organs, such as the liver and spleen, had the power of converting oxypurines into uric acid in the presence of oxygen (HORBACZEWSKI, SPITZER and WIENER [3]), recently SCHITTENHELM, BURIAN, JONES and PARTRIDGE,[4] by more careful investigations have shown that enzymes of different kinds act together. By means of the two deamidizing enzymes *adenase* and *guanase* the adenine and guanine are transformed into hypoxanthine and xanthine respectively, and from the latter by means of an oxidizing enzyme, called *xanthine oxidase* by BURIAN, the uric acid is formed. In the formation of uric acid from the nucleoproteins we must admit of a gradual decomposition of these by the aid of different enzymes, proteases, nucleases and deamidases. The deamidases seem to be present in most organs, and we have numerous investigations upon their distribution, especially those of JONES and SCHITTENHELM and

[1] Pflüger's Arch., **80**, **87**, and **94**.

[2] Sivén, l. c.; Rockwood, Amer. Journ. of Physiol., **12**.

[3] See footnote 6, page 666.

[4] Schittenhelm, Zeitschr. f. physiol. Chem., **42**, **43**, **45**, **46**, **57**, with Schmid, *ibid.*, **50** and Zeitschr. f. exp. Path. u. Therap., **4**; Burian, Zeitschr. f. physiol. Chem., **43**; Jones and Partridge, *ibid.*, **43**; Jones with Winternitz, *ibid.*, **44** and **60**; Jones, *ibid.*, **45**, with Austrian, *ibid.*, **48**.

his collaborators.[1] The distribution is not the same in all animals and the reports regarding it are conflicting. SCHITTENHELM indeed claims that it has not been proven that guanase and adenase are two different enzymes. Still we have other grounds for the non-identity of the two amidases.

In birds the conditions are different. v. MACH[2] has shown that in the bird family a part of the uric acid may be formed from the purine bodies. The chief quantity of uric acid, however, is undoubtedly formed in birds by synthesis.

The formation of uric acid in birds is increased by the administration of ammonium salts (v. SCHRÖDER), and urea acts in a similar manner (MEYER and JAFFÉ). MINKOWSKI observed, in geese with extirpated livers, a very significant decrease in the elimination of uric acid, while the elimination of ammonia was increased to a corresponding degree. This indicates a participation of ammonia in the formation of uric acid in the organism of birds; and as MINKOWSKI has also found, after the extirpation of the liver, that considerable amounts of lactic acid occur in the urine, it is probable that the uric acid in birds is produced in the liver by synthesis, perhaps from lactic acid and ammonia; although, as SALASKIN and ZALESKI and LANG have shown, after the extirpation of the liver, an increase in the formation of lactic acid primarily occurs, and this causes an increase in the elimination of ammonia (neutralization ammonia). The direct proof for the uric-acid formation from ammonia and lactic acid in the liver of birds has been given by KOWALEWSKI and SALASKIN[3] by means of blood-transfusion experiments on geese with extirpated livers. They observed a relatively abundant formation of uric acid after the addition of ammonium lactate and a still greater formation after arginine. They not only consider ammonium lactate but also amino-acids as substances from which the uric acid can be produced in the liver by synthesis. That these, for example, leucine, glycocoll, and aspartic acid, increase the elimination of uric acid in birds was first shown by v. KNIERIEM.[4]

The possibility of a formation of uric acid from lactic acid has been shown in another manner by WIENER,[5] namely, by feeding birds with urea and lactic acid and different non-nitrogenous substances, oxy-,

[1] See footnote 4, p. 667. See also Mendel and Mitchell, Amer. Journ. of Physiol., **20**.

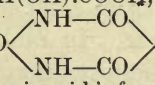
[2] Arch. f. exp. Path. u. Pharm., **24**.

[3] v. Schröder, Zeitschr. f. physiol. Chem., **2**; Meyer and Jaffé, Ber. d. d. Chem. Gesellsch., **10**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **29**; Lang, *ibid.*, **32**; Kowalewski and Salaskin, *ibid.*, **33**.

[4] Zeitschr. f. Biologie, **13**.

[5] Hofmeister's Beiträge, **2**. See also Arch. f. exp. Path. u. Pharm., **42**, and Ergebnisse d. Physiol., **1**, Abt. 1, 1902.

keto-, and dibasic acids of the aliphatic series. The dibasic acids, with a chain of 3 carbon atoms or their ureides, showed themselves most active as uric-acid formers, and WIENER is therefore of the opinion that the active substances must first be converted into dibasic acids. By the attachment of a urea residue the corresponding ureide is produced, according to WIENER, and from this the uric acid is derived by the attachment of a second urea residue.

Among the substances tested, only tartronic acid and its ureide, dialuric acid, have shown themselves active in the experiments with the isolated organs, and WIENER therefore also considers that the other acids must be first converted into tartronic acid by oxidation or reduction. From lactic acid, $CH_3.CH(OH).COOH$, we first obtain tartronic acid, $COOH.CH(OH).COOH$, which by the attachment of a urea residue forms dialuric acid,  , and from this, by the attachment of a second urea residue, uric acid is formed.

In opposition to the above-mentioned observations and opinions we must remark that recently FRIEDMANN and H. MANDEL,[1] by transfusion experiments with geese livers, have come to other conclusions. They found an increase in the uric acid content in the transfused blood without adding any substance, but found no increase in the quantity of uric acid when the transfused blood was treated with urea alone or with urea and sodium lactate or malonate. Further research must explain this contradiction.

We cannot give any positive answer as to the question whether uric acid is formed by synthesis in man and other mammalia. WIENER has reported experiments which seem to indicate a synthetic uric-acid formation in the isolated mammalian liver, and he has also obtained an increase in the uric-acid elimination, although only a slight one, after feeding lactic acid and dialuric acid to man. In opposition to these experiments PFEIFFER[2] could find no increase in the elimination of uric acid after feeding malonamide and tartronamide to monkeys as well as tartronic acid and pseudouric acid to monkeys or human beings, and he finds that a synthesis of uric acid in mammalia and man is very doubtful. According to BURIAN[3] we have for the present no proof of a synthetical formation of uric acid in the mammalian liver. Dialuric acid and tartronic acid, according to him, do not cause any marked uric-acid formation with extracts of the ox-liver in the absence of purine bases; on the contrary they accelerate the enzymotic oxidation of purine bases and hence, according to BURIAN, this explains, perhaps, the increase in uric-acid elimination.

The liver seems to be the organ in birds where the synthetical formation of uric acid occurs, and the fact that it was possible for MINKOWSKI[4]

[1] Arch. f. exp. Path. u. Pharm., 1908; Suppl. Schmiedeberg's Festschrift.

[2] Hofmeister's Beiträge, **10.**

[3] Zeitschr. f. physiol. Chem., **43.**

[4] l. c.

to arrest the uric-acid formation by the extirpation of the liver, apparently shows that the liver is the only organ taking part in this synthesis. If a synthesis of uric acid also occurs in man and other mammalia, we must consider the liver as at least one of the organs taking part in the work, as shown by WIENER'S investigations. The liver, spleen, and muscles are considered as the most important organs in the oxidative formation of uric-acid from nucleins and purine bases, but it must not be forgotten that these organs in different animals behave dissimilarly in this regard.

Uric acid when introduced into the mammalian organism is, as first shown by WÖHLER and FRERICHS, in the dog, and later substantiated by several experimenters,[1] in great part destroyed and more or less completely changed into urea. In rabbits, according to WIENER, the uric acid is destroyed with the formation of glycocoll as an intermediate step. HIRSCHSTEIN has recently accepted this view, but WIECHOWSKI, SAMUELY, BRUGSCH and SCHITTENHELM[2] dispute it. Still we must consider the earlier opinion of WÖHLER and FRERICHS as probably correct, that allantoin is the chief intermediary product. This is based upon the work of SALKOWSKI, MENDEL and BROWN, MENDEL and WHITE, and WIECHOWSKI, but it is denied by WIENER, POHL and PODUSCHKA. The intermediary transformation of uric acid into allantoin occurs, in WIECHOWSKI'S[3] opinion, only in the mammals investigated, but not in man.

The demolition of uric acid seems to be possible, according to the numerous researches of CHASSEVANT and RICHET, ASCOLI, JACOBY, WIENER, SCHITTENHELM, BURIAN, ALMAGIA, PFEIFFER, and WIECHOWSKI, in several organs, such as the liver, kidneys, muscle, and bone-marrow, although this behavior differs in various animals. This destruction of uric acid, which is called *uricolysis*, is an enzymotic process. WIECHOWSKI and WIENER[4] have made careful investigations on the active

[1] Wöhler and Frerichs, Annal. d. Chem. u. Pharm., **65**. See also Wiener, Ergebnisse der Physiologie, **1**, Abt. 1.

[2] Hirschstein, Zeitschr. f. exp. Path. u. Ther., **4**, and Arch. f. exp. Path. u. Pharm., **59**; Wiechowski, Hofmeister's Beiträge, **9**; Samuely, Zeitschr. f. exp. Path. u. Ther., **4**; Brugsch and Schittenhelm, *ibid.*, **4**.

[3] Wiener, Arch. f. exp. Path. u. Pharm., **40** and **42**, and Ergebnisse der Physiologie, **1**, Abt. 1; Pohl, Arch. f. exp. Path. u. Pharm., **48**; Poduschka, *ibid.*, **44**; Salkowski, Zeitschr. f. physiol. Chem., **35**, and Ber. d. d. Chem. Gesellsch., **9**; Mendel and Brown, Amer. Journ. of Physiol., **3**; Mendel and White, *ibid.*, **12**; Wiechowski, Arch. f. exp. Path. u. Pharm., **60**, with Wiener, Hofmeister's Beiträge, **9**. Conflicting reports in Croftan, Pflüger's Arch., **121**.

[4] Chassevant and Richet, Comp. rend. soc. biolog., **49**; Ascoli, Pflüger's Arch., **72**; Jacoby, Virchow's Arch., **157**; Wiener, Arch. f. exp. Path. u. Pharm., **42**, and Centralbl. f. Physiol., **18**; Schittenhelm, Zeitschr. f. physiol. Chem., **43** and **45**; Burian, *ibid.*, **43**; Almagia, Hofmeister's Beiträge, **7**; Pfeiffer, *ibid.*, **7**; Wiechowski and Wiener, *ibid.*, **9**.

enzyme of the ox-kidney and dog-liver and find that it is an oxidase only active in faintly alkaline or neutral reaction. WIECHOWSKI has also found that this enzyme transforms the uric acid into allantoin almost entirely. ASCOLI and IZAR[1] have also made the remarkable observation that if an extract of liver which has completely destroyed a known quantity of uric acid (air or oxygen being passed through) is allowed to stand for some time in the thermostat, with the exclusion of air, the destroyed uric acid is gradually reformed again. It has not been determined from what transformation products this is produced. Still it is very suggestive in connection with WIECHOWSKI's reports on the formation of allantoin that the allantoin is inactive in this regeneration of the uric acid. Further enlightenment on this point would be of the greatest interest.

From this power of the various organs of destroying uric acid it follows that the quantity of uric acid eliminated is not a sure indication of the amount of the acid formed. We must, therefore, admit that a part of the uric acid formed in the body is destroyed in a manner similar to that introduced from without. BURIAN and SCHUR[2] have indeed suggested a factor, the so-called "integral factor," with which the quantity of uric acid eliminated in the twenty-four hours must be multiplied in order to find the quantity of uric acid formed during this time. According to them, carnivora eliminate unchanged about $\frac{1}{20} - \frac{1}{30}$ of the uric acid introduced into the circulation, rabbits about $\frac{1}{6}$, and man $\frac{1}{2}$. Such calculations are necessarily very uncertain, and for man, in whom according to WIECHOWSKI practically no intermediary destruction of uric acid occurs, they are for the present not admissible.

Properties and Reactions of Uric Acid. Pure uric acid is a white, odorless, and tasteless powder consisting of very small rhombic prisms or plates. Impure uric acid is easily obtained as somewhat larger, colored crystals.

In rapid crystallization, small, thin, four-sided, apparently colorless, rhombic prisms are formed, which can be seen only by the aid of the microscope, and these sometimes appear as spools because of the rounding of their obtuse angles. The plates are sometimes six-sided, irregularly developed; in other cases they are rectangular with partly straight and partly jagged sides; and in other cases they show still more irregular forms, the so-called dumb-bells, etc. In slow crystallization, as when the urine deposits a sediment or when treated with acid, large, invariably colored crystals separate. Examined with the microscope these crystals always appear yellow or yellowish brown in color. The most common type is the whetstone shape, formed by the rounding off of the obtuse

[1] Zeitschr. f. physiol. Chem., 58.

[2] Pflüger's Arch., 87.

angles of the rhombic plate. The whetstones are generally connected, two or more crossing each other. Besides these forms, rosettes of prismatic crystals, irregular crosses, brown-colored rough masses of broken-up crystals and prisms occur, as well as other forms.

Uric acid is insoluble in alcohol and ether; it is rather easily soluble in boiling glycerin, but very insoluble in cold water, in 39480 parts at 18° C. (His and Paul), and in 15505 parts at 37° (Gudzent). At this temperature, according to His and Paul, 9.5 per cent of the uric acid is dissociated in the saturated solution. Because of the reduction in the dissociation on the addition of strong acids, uric acid is soluble with difficulty in the presence of mineral acids. It is soluble in a warm solution of sodium diphosphate, and in the presence of an excess of uric acid, monophosphate and acid urate are produced. It is ordinarily assumed that sodium diphosphate forms a solvent for the uric acid in the urine, but Smale claims that the monophosphate has only a slight solvent action. Rüdel [1] believes that urea is an important solvent, but this view has not been confirmed by the observations of His and Paul. Uric acid is not only dissolved by alkalies and alkali carbonates, but also by several organic bases, such as ethylamine and propylamine, piperidine and piperazine. Uric acid dissolves, without decomposing, in concentrated sulphuric acid. It is completely precipitated from the urine by picric acid (Jaffé [2]). Uric acid gives a chocolate-brown precipitate with phosphotungstic acid in the presence of hydrochloric acid.[3]

Uric acid is dibasic and consequently forms two series of salts, neutral and acid. Of the alkali urates the lithium salts are the most soluble and the acid ammonium salt is the most insoluble. The acid alkali urates are very insoluble and separate as a sediment (*sedimentum lateritium*) from concentrated urine on cooling. According to Gudzent 1 liter of water at 18° C. dissolves (as primary salts) 1.5313 grams potassium, 0.8328 grams sodium, and 0.4141 grams ammonium urate, and at 37° C. 2.7002, 1.5043 and 0.7413 grams of the respective urates. The salts of the alkaline earths are soluble with great difficulty. The above solubilities apply only, in Gudzent's [4] experience, to the freshly prepared solution, as the solubility to a certain limit gradually diminishes, due to intramolecular transposition (change of the uric acid from the lactam-form into the lactim-form).

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[1] His, Jr., and Paul, Zeitschr. f. physiol. Chem., **31**; Smale, Centralbl. f. physiol., **9**; Rüdel, Arch. f. exp. Path. u. Pharm., **30**; Gudzent, Zeitschr. f. physiol. Chem., **60**.

[2] Zeitschr. f. pyhsiol. Chem., **10**.

[3] In regard to the combinations of formaldehyde and uric acid, see Nicolaier, Deutsch. Arch. f. klin. Med., **89** (1906).

[4] Zeitschr. f. physiol. Chem., **56** and **60**.

If a little uric acid in substance is treated on a porcelain dish with a few drops of nitric acid, the uric acid dissolves on warming, with a strong development of gas, and after thoroughly drying on the water-bath a beautiful red residue is obtained, which turns a purple-red (ammonium purpurate or murexide) on the addition of a little ammonia. If instead of the ammonia we add a little caustic soda (after cooling), the color becomes deeper blue or bluish violet. This color disappears quickly on warming, differing from certain purine bodies. This reaction is called the *murexide test.*

If uric acid is converted into alloxan by the careful action of nitric acid and the excess of acid carefully expelled, on treating this with a few drops of concentrated sulphuric acid and commercial benzene (containing thiophene) a beautiful blue coloration is obtained (DENIGÈS' reaction[1]).

Uric acid does not reduce an alkaline solution of bismuth, while, on the contrary, it reduces an alkaline cupric-hydroxide solution. In the presence of only a little copper salt we obtain a white precipitate consisting of cuprous urate. In the presence of more copper salt red cuprous oxide separates. The compound of uric acid with cuprous oxide is formed when copper salts are reduced by dextrose or a bisulphite in alkaline solution in the presence of a sufficient amount of urate.

If a solution of uric acid in water containing alkali carbonate is treated with magnesium mixture and then a silver-nitrate solution added, a gelatinous precipitate of silver-magnesium urate is formed. If a drop of uric acid dissolved in sodium carbonate is placed on a piece of filter-paper which has been previously treated with silver-nitrate solution, a reduction of silver oxide occurs, producing a brownish-black or, in the presence of only 0.002 milligram of uric acid, a yellow spot (SCHIFF'S test).

If a weak alkaline solution of uric acid in water is treated with a soluble zinc salt, a white precipitate is produced, which on the filter in the presence of alkali is oxidized by the air, and becomes sky-blue in color, especially in sunlight. Potassium persulphate causes a blue coloration immediately (GANASSINI'S reaction[2]).

The precipitation of free uric acid from its alkali salts by means of acids can be prevented to some extent by the presence of thymic acid or nucleic acid (GOTO). According to SEO[3] we are here dealing with combinations of 1 molecule nucleic acid and 2 molecules uric acid, which protects the uric acid within the body against destruction or transformation into allantoin.

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[1] Journ. de Pharm. et de Chim., 18. Cited from Maly's Jahresber., 18.

[2] Cited f. Bioch. Cenetralbl., 8, 250.

[3] Goto, Zeitschr f. physiol. Chem., 30; Seo, Arch. f. exp. Path. u. Pharm., 58.

Preparation of Uric Acid from Urine. Filtered normal urine is treated with 20–30 cc. of 25-per cent hydrochloric acid for each liter of urine. After forty-eight hours collect the crystals and purify them by redissolving in dilute alkali, decolorizing with animal charcoal and reprecipitating with hydrochloric acid. Large quantities of uric acid are easily obtained from the excrement of serpents by boiling it with dilute caustic potash (5-per cent) until no more ammonia is developed. A current of carbon dioxide is passed through the filtrate until it barely has an alkaline reaction; dissolve the separated and washed acid potassium urate in caustic potash, and precipitate the uric acid in the filtrate by addition of an excess of hydrochloric acid.

Quantitative Estimation of Uric Acid in the Urine. As the older method suggested by HEINTZ, even after recent modifications, gives inaccurate results, it will not be considered here.

SALKOWSKI and LUDWIG'S [1] method consists in precipitating the uric acid, by silver nitrate, from the urine previously treated with magnesium mixture, and weighing the uric acid obtained from the silver precipitate. Uric acid determinations by this method are often performed according to the suggestion of E. LUDWIG, which requires the following solutions:

1. An AMMONIACAL SILVER-NITRATE SOLUTION, which contains in 1 liter 26 grams of silver nitrate and a quantity of ammonia sufficient to redissolve completely the preciptate produced by the first addition of ammonia. 2. MAGNESIA MIXTURE. Dissolve 100 grams of crystallized magnesium chloride in water, add ammonia until the liquid smells strongly of it, and enough ammonium chloride to dissolve the precipitate; then dilute the solution to 1 liter. 3. SODIUM SULPHIDE SOLUTION. Dissolve 10 grams of caustic soda which is free from nitric acid and nitrous acid in 1 liter of water. One half of this solution is completely saturated with sulphuretted hydrogen and then mixed with the other half.

The concentration of the three solutions is so arranged that 10 cc. of each is sufficient for 100 cc. of the urine.

100–200 cc., according to concentration, of the filtered urine, freed from protein (by boiling after the addition of a few drops of acetic acid), are poured into a beaker. In another vessel mix 10–20 cc. of the silver solution with 10–20 cc. of the magnesia mixture and add ammonia, and when necessary also some ammonium chloride, until the mixture is clear. This solution is added to the urine while stirring, and the mixture allowed to stand quietly for half an hour. The precipitate is collected on a filter, washed with ammoniacal water, and then returned to the same beaker by the aid of a glass rod and a wash-bottle, without destroying the filter. Now heat to boiling 10–20 cc. of the alkali-sulphide solution, which has previously been diluted with an equal volume of water, and allow this solution to flow through the above filter into the beaker containing the silver precipitate; wash with boiling water, and warm the contents of the beaker on a water-bath for a time, stirring constantly. After cooling, filter into a porcelain dish, wash the filter with boiling water, acidify the filtrate with hydrochloric acid, evaporate

[1] Salkowski, Virchow's Arch., 52; Pflüger's Arch., 5; Salkowski, Laboratory Manual of Physiol. and Path. Chem., translated by Orndorff, 1904; Ludwig, Wien. med. Jahrbuch, 1884, and Zeitschr f anal Chem , 24.

it to about 15 cc., add a few drops more of hydrochloric acid, and allow it to stand for twenty-four hours. The uric acid which has crystallized is collected on a small weighed filter, washed with water, alcohol, ether, and carbon disulphide, dried at 100–110° C., and weighed. For each 10 cc. of aqueous filtrate we must add 0.00048 gram uric acid to the quantity found directly. Instead of the weighed filter-paper a glass tube filled with glass wool as described in other handbooks may be substituted (Ludwig). Too intense or too long continued heating with the alkali sulphide must be prevented, otherwise a part of the uric acid may be decomposed.

Salkowski deviates from this procedure by first precipitating the urine with a magnesium mixture (50 cc. to 200 cc. urine), filling up to 300 cc., and filtering. Of the filtrate, 200 cc. are precipitated by 10–15 cc. of a 3-per cent silver-nitrate solution. The silver precipitate is shaken with 200–300 cc. of water acidified with a few drops of hydrochloric acid, decomposed by sulphuretted hydrogen, heated to boiling, the silver-sulphide precipitate boiled with fresh water, filtered, the filtrate concentrated to a few cubic centimeters, treated with 5–8 drops of hydrochloric acid, and allowed to stand until the next day.

Hopkins' method is based on the fact that the uric acid is completely precipitated from the urine as ammonium urate on saturating with ammonium chloride. The uric acid can either be weighed after being set free by hydrochloric acid or it can be determined in several ways—by titration with potassium permanganate or by the Kjeldahl method. Several modifications of this method have been worked out by Folin, Folin and Schaffer, Wörner, and Jolles.[1] The last named converts the uric acid into urea by oxidation with potassium permanganate in sulphuric-acid solution and then determines the quantity of this by sodium hypobromite. Of these methods we shall describe only that suggested by Folin-Schaffer.

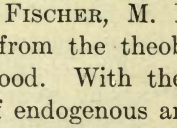
Folin-Schaffer Method. Treat 300 cc. urine with 75 cc. of a solution containing 500 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 cc. of 10-per cent acetic acid in a liter, and filter after five minutes. This removes an unknown constituent of the urine (a protein substance) which would otherwise contaminate the uric acid. Take 125 cc. of the filtrate (corresponding to 100 cc. of the urine) and add 5 cc. of concentrated ammonia. After twenty-four hours the precipitate is filtered off and washed free from chlorine on the filter by means of an ammonium-sulphate solution. The precipitate is washed off the filter by water (total 100 cc.) into a flask, treated with 15 cc. of concentrated sulphuric acid, and titrated at 60–63° C. with N/20 potassium-permanganate solution. Each cubic centimeter of this solution corresponds to 3.75 milligrams uric acid. Because of the solubility of the ammonium urate a correction of 3 milligrams must be added for every 100 cc. of the urine.

In regard to the numerous other methods for estimating uric acid, we must refer to special works on the subject, and especially to Huppert-Neubauer.

[1] Hopkins, Journ. of Path. and Bact., 1893, and Proceed. Roy. Soc., 52; Folin, Zeitschr. f. physiol. Chem., 24; Folin and Schaffer, *ibid.*, 32; Wörner, *ibid.*, 29; Jolles, *ibid.*, 29, and Wien. med. Wochenschr., 1903.

Purine Bases (Alloxuric Bases). The purine bases found in human urine are *xanthine, guanine, hypoxanthine, adenine, paraxanthine, heteroxanthine, episarkine, epiguanine,* 1-*methylxanthine,* and *carnine.* The occurrence of guanine and carnine (Pouchet) is, according to Krüger and Salomon,[1] not positively shown. The quantity of these bodies in the urine is extremely small and varies in different individuals. Flatow and Reitzenstein [2] found 15.6–45.1 milligrams in the urine voided during twenty-four hours. The quantity of alloxuric bases in the urine is regularly increased after feeding with nucleins or food rich in nucleins, and after an abundant destruction of leucocytes. The quantity is especially increased in leucæmia. We have a number of observations on the elimination of these bodies in different diseases, but they are hardly trustworthy on account of the inaccuracy of the methods used in the determinations. It must also be remarked that the three purine bases, heteroxanthine, paraxanthine, and 1-methylxanthine, which form the chief mass of the purine bases of the urine, are derived, according to the investigations of Albanese, Bondzynski and Gottlieb, E. Fischer, M. Krüger and G. Salomon, and Schmidt and Kotake [3] from the theobromine, caffeine, and theophylline which occur in the food. With the purine bases we must also differentiate between those of endogenous and those of exogenous origin,[4] and the same factors apply as for the uric acid, viz., the endogenous purine formation represents a value which is somewhat variable for different individuals and relatively constant for the same individual. According to Sivén,[5] with purine-free diet the elimination of purines is lowest at night and highest in the morning hours. Rest and work do not show any positive difference. As the four true nuclein bases and carnine have been treated in Chapters III and XI, it only remains to describe the special urinary purine bodies.

Heteroxanthine, $C_6H_6N_4O_2$, 7-monomethylxanthine,



was first

[1] Zeitschr. f. physiol. Chem., **24**; Pouchet, "Contributions à la connaissance des matières extractives de l'urine." Thèse Paris, 1880. Cited from Huppert-Neubauer, 333 and 335.

[2] Deutsch. med. Wochenschr., 1897.

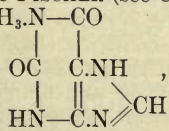
[3] Albanese, Ber. d. d. chem. Gesellsch., **32**; Arch. f. exp. Path. u. Pharm., **35**; Bondzynski and Gottlieb, *ibid.*, **36**, and Ber. d. deutsch. chem., Gesellsch., **28**; E. Fischer, *ibid.*, **30**, 2405; Krüger and Salomon, Zeitschr. f. physiol. Chem., **26**; Krüger and Schmidt, Ber. d. d. chem. Gesellsch., **32**, and Arch. f. exp. Path. u. Pharm., **45**; Kotake, Zeitschr. f. physiol. Chem., **57**.

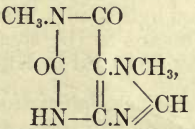
[4] See Burian and Schur, footnote 1, page 667, and Kaufmann and Mohr, Deutsch. Arch. f. klin. Med., **74**.

[5] Skand. Arch. f. Physiol., **18**.

detected in the urine by SALOMON. It is identical with the monomethylxanthine which passes into the urine after feeding with theobromine or caffeine. SALOMON and NEUBERG [1] found heteroxanthine in the urine of a dog fed entirely upon meat, and this was probably formed by a methylation in the body.

Heteroxanthine crystallizes in shining needles and dissolves with difficulty in cold water (1592 parts at 18° C.). It is readily soluble in ammonia and alkalies. The crystalline sodium salt is insoluble in strong caustic alkali (33-per cent) and dissolves with difficulty in water. The chloride crystallizes beautifully, is relatively insoluble, and is readily decomposed into the free base and hydrochloric acid by water. Heteroxanthine is precipitated by copper sulphate and bisulphite, mercuric chloride, basic lead acetate and ammonia, and by silver nitrate. The silver compound dissolves rather easily in dilute, warm nitric acid; it crystallizes in small rhombic plates or prisms, often grown together, forming characteristic crosses. Heteroxanthine does not give the xanthine reaction, but does give WEIDEL'S reaction, according to FISCHER (see Chapter III).

1-Methylxanthine, $C_6H_6N_4O_2$,  , was first isolated from the urine and studied by KRÜGER, and then by KRÜGER and SALOMON.[2] It is difficultly soluble in cold water, but readily soluble in ammonia and caustic soda, and does not give an insoluble sodium compound. It is readily soluble in dilute acids, and it crystallizes from its acetic-acid solution in thin, generally hexagonal plates. The chloride is decomposed into the base and hydrochloric acid by water. 1-methylxanthine gives crystalline double salts with platinum and gold. It is not precipitated by basic lead acetate, nor when pure by basic lead acetate and ammonia. With ammonia and silver nitrate it gives a gelatinous precipitate. The silver-nitrate compound crystallized from nitric acid forms rosettes of united needles. With the xanthine test with nitric acid it gives an orange coloration on the addition of caustic soda. It gives WEIDEL'S reaction (according to FISCHER) beautifully.

Paraxanthine, $C_7H_8N_4O_2$, . 1.7-dimethylxanthine,  , *urotheobromine* (THUDICHUM), was first isolated from the urine by THUDICHUM and SALOMON.[3] It crystallizes beautifully in six-sided plates or in needles. The sodium compound crystallizes in rectangular plates or prisms and, like the heteroxanthine-sodium compound, is insoluble in 33-per cent caustic-soda solution. The sodium compound separates in a crystalline state on neutralizing its solution in water. The chloride is readily soluble and is not decomposed by water. The chloroplatinate crystallizes very beautifully. Mercuric chloride precipitates it only when added in excess and after a long time. The silver-nitrate compound separates as white silky crystals from hot nitric acid on cooling. It gives WEIDEL'S reaction, but not the xanthine test, with nitric acid and alkali.

Episarkine is the name given by BALKE to a purine body occurring in human urine. The same body has been observed by SALOMON [4] in pigs' and dogs' urine,

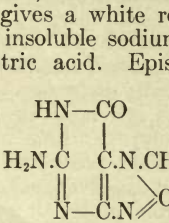
[1] Salkowski's Festschrift, Berlin, 1904.

[2] Krüger, Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f. physiol. Chem., **24**.

[3] Thudichum, "Grundzüge d. anal. med. klin. Chemie" (Berlin, 1886); Salomon, Arch. f. (Anat. u.) Physiol., 1882, and Ber. d. deutsch. chem. Gesellsch., **16** and **18**.

[4] Balke, "Zur Kenntniss der Xanthinkörper" (Inaug.-Diss., Leipzig, 1893); Salomon, Zeitschr. f. physiol. Chem., **18**.

as well as in urine in leucæmia. BALKE gives $C_4H_6N_3O$ as the probable formula for episarkine. It is nearly insoluble in cold water, dissolves with difficulty in hot water, but may be obtained therefrom as long fine needles. Episarkine does not give the xanthine reaction with nitric acid, or WEIDEL'S reaction. With hydrochloric acid and potassium chlorate it gives a white residue which turns violet with ammonia. It does not form any insoluble sodium compound. The silver compound is difficultly soluble in nitric acid. Episarkine is possibly identical with epiguanine.

Epiguanine, $C_6H_7N_5O$, 7-methylguanine,  , was first prepared from the urine by KRÜGER.[1] It is crystalline and difficultly soluble in hot water or ammonia. It crystallizes from a hot 33-per cent caustic-soda solution on cooling in broad shining crystals and dissolves readily in hydrochloric or sulphuric acid. It gives a characteristic chloroplatinate crystallizing in six-sided prisms. It is precipitated neither by basic lead acetate nor by basic lead acetate and ammonia. Silver nitrate and ammonia give a gelatinous precipitate. It responds to the xanthine test with nitric acid and alkali. Acts like episarkine with WEIDEL'S test according to FISCHER.

In preparing alloxuric bases from the urine, the fluid is supersaturated with ammonia and precipitated by a silver-nitrate solution. The precipitate is then decomposed with sulphuretted hydrogen. The boiling-hot filtrate is evaporated to dryness and the dried residue treated with 3-per cent sulphuric acid. The purine bases are dissolved, while the uric acid remains undissolved. This filtrate is saturated with ammonia and precipitated by silver-nitrate solution. If instead of precipitating with silver solution we desire to precipitate, according to KRÜGER and WULFF, with copper suboxide, the urine may be heated to boiling, and immediately are added, successively, 100 cc. of a 50-per cent sodium-bisulphite solution and 100 cc. of a 12-per cent copper-sulphate solution for every liter of urine. The thoroughly washed precipitate is decomposed with hydrochloric acid and sulphuretted hydrogen. The uric acid remains in great part on the filter. Further details in regard to the treatment of the solution of the hydrochloric-acid compounds may be found in KRÜGER and SALOMON.[2]

Quantitative Estimation of Purine Bases according to SALKOWSKI.[3] 400–600 cc. of the urine free from protein are first precipitated by magnesia mixture and then by a 3-per cent silver-nitrate solution as described on page 675. The thoroughly washed silver precipitate is decomposed by sulphuretted hydrogen after being suspended in 600–800 cc. of water with the addition of a few drops of hydrochloric acid. It is heated to boiling and filtered hot, and finally evaporated to dryness on the water-bath. The residue is extracted with 20–30 cc. of hot 3-per cent sulphuric acid and allowed to stand twenty-four hours; the uric acid is filtered off, washed, the filtrate made ammoniacal, and the purine bodies again precipitated by silver nitrate, the precipitate collected on a small chlorine-free filter, washed thoroughly, dried, carefully incinerated, the ash dissolved in nitric acid, and titrated with ammonium sulpho-

[1] Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f. physiol. Chem., **24** and **26**.

[2] Zeitschr. f. physiol. Chem., **26**, and also Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl., 188.

[3] Pflüger's Arch., **69**.

cyanide according to VOLHARD's method. The ammonium-sulphocyanide solution should contain 1.2–1.4 grams per liter, and its strength should be determined by a silver-nitrate solution: 1 part silver corresponds to 0.277 gram nitrogen of purine bases or to 0.7381 gram purine bases. By this method the uric-acid and purine bases can be simultaneously determined in the same portion of urine.[1]

MALFATTI[2] determines the nitrogen of the purine bases in the hydrochloric-acid filtrate from the separated uric acid. This filtrate is evaporated with magnesia until all the ammonia has been expelled and the residue used for the KJELDAHL determination.

The nitrogen of the purine bases is also determined as the difference between the uric-acid nitrogen and the total nitrogen of the purine bodies of the silver precipitate (CAMERER, ARNSTEIN[3]). SALKOWSKI has raised the objection to this procedure that it is not possible to remove all the ammonia from the silver precipitate by washing. According to ARNSTEIN[4] this can readily be done by boiling the precipitate in water with some magnesia, and under these circumstances this method is quite serviceable. The nitrogen is estimated by KJELDAHL's method. The uric-acid nitrogen multiplied by 3 gives the quantity of uric acid. As the mixture of purine bases in the urine is but little known, the quantity of nitrogen of the purine bases is always calculated as a certain purine base, for example xanthine (CAMERER), and the quantity so found used as a measure for the purine bases.

According to a new method of KRÜGER and SCHMID[5] the uric acid and the purine bases are precipitated as a cuprous compound by copper-sulphate solution and sodium bisulphite. The precipitate is decomposed in sufficient water by sodium sulphide, and the uric acid precipitated from the concentrated filtrate with hydrochloric acid, and the purine bases again precipitated from this filtrate as cuprous or silver compounds. Finally, the nitrogen in the uric-acid part and the part containing the mixture of purine bases is estimated.

We cannot discuss the other methods, such as those of DENIGÈS and NIEMILOWICZ, and the method suggested by HALL[6] for clinical purposes.

Oxaluric Acid, $C_3H_4N_2O_4 = (CON_2H_3).CO.COOH$. This acid, whose relation to uric acid and urea has been spoken of above, does not always occur in the urine, and then only in traces as the ammonium salt. This salt is not directly precipitated by $CaCl_2$ and NH_3, but on boiling it is decomposed into urea and oxalate. In preparing oxaluric acid from urine the latter is filtered through animal charcoal. The oxaluric retained by the charcoal may be obtained by boiling with alcohol.

Oxalic Acid, $C_2H_2O_4$, or $\begin{matrix} COOH \\ | \\ COOH \end{matrix}$, occurs under physiological conditions in very small amounts in the urine, about 0.02 gram in twenty-four hours (FÜRBRINGER[7]). According to the generally accepted view it exists in

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[1] In regard to the details we refer the reader to the original paper.

[2] Centralbl. f. innere Med., 1897.

[3] Camerer, Zeitschr. f. Biologie, **26** and **28**; Arnstein, Zeitschr. f. physiol. Chem., **23**.

[4] Salkowski, l. c.; Arnstein, Centralbl. f. d. med. Wissensch., 1898.

[5] Zeitschr. f. physiol. Chem., **45** and Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl., 590.

[6] Niemilowicz, Zeitschr. f. physiol. Chem., **35**; Gittelmacher-Wilenko, *ibid.*, **36**; Hall, Wien. klin. Wochenschr., **16**.

[7] Deutsch. Arch. f. klin. Med., **18**. See also Dunlop, Journ. Path. and Bacteriol., **3**.

the urine as calcium oxalate, which is kept in solution by the acid phosphates present. Calcium oxalate is a frequent constituent of urinary sediments, and also occurs in certain urinary calculi.

The origin of the oxalic acid in the urine is not well known. Oxalic acid when administered is eliminated unchanged, at least in part, by the urine;[1] and as many vegetables and fruits, such as cabbage, spinach, asparagus, sorrel, apples, grapes, etc., contain oxalic acid, it is possible that a part of the oxalic acid of the urine originates directly from the food. That oxalic acid may be formed in the animal body as a metabolic product from proteins or fats follows from the observations of Mills and Lüthje,[2] who found that in dogs on an exclusively meat and fat diet, as also in starvation, oxalic acid was eliminated by the urine. The oxalic acid which is eliminated in increased quantity with a diminished oxygen supply and an increased protein catabolism, as found by Reale and Boeri, and also by Terray, is supposed to be derived partly from the greater destruction of proteins. Pure protein does not, according to Salkowski,[3] increase the quantity of oxalic acid eliminated; on the contrary, after meat feeding the amount of this acid is increased, due in part to the meat containing oxalic acid (Salkowski). Gelatin and gelatin-yielding tissues seem to increase the excretion of oxalic acid, which stands in accord with the observations of Kutscher and Schenck[4] that on the oxidation of gelatin oxamic acid is produced from the glycocoll and this then readily decomposes into ammonia and oxalic acid. After feeding nucleins no constant increase in the elimination of oxalic acid has been observed.[5] The production of oxalic acid due to an incomplete combustion of the carbohydrates has also been suggested. The work of Hildebrandt and P. Mayer seems to indicate this under abnormal conditions. In alimentary glycosuria or diabetes Luzzato[6] could not observe any rise in the elimination of oxalic acid. According to Dakin,[7] in rabbits an increased elimination of oxalic acid occurs after the introduction of glycollic or glyoxylic acids, and the oxalic acid seems to be an intermediary product of metabolism, which is further burnt. We cannot exclude the possibility of the formation of oxalic acid in

[1] In regard to the behavior of oxalic acid in the animal body, see page 733.

[2] Mills, Virchow's Arch., **99**; Lüthje, Zeitschr. f. klin. Med., **35**.

[3] Reale and Boeri, Wien. med. Wochenschr., 1895; Terray, Pflüger's Arch., **65**; Salkowski, Berl. klin. Wochenschr., 1900.

[4] Zeitschr. f. physiol. Chem., **43**.

[5] See Stradomsky, Virchow's Arch., **163**; Mohr and Solomon, Deutsch. Arch. f. klin. Med., **70**; Salkowski, l. c.

[6] Hildebrandt, Zeitschr. f. physiol. Chem., **35**; P. Mayer, Zeitschr. f. klin. Med., **47**; Luzzato, Salkowski's Festschrift, 1904.

[7] Journ. of Biol. Chem., **3**, 57.

the oxidation of uric acid in the animal body, yet we have no positive proof of such a formation.[1]

Oxalic acid is best detected and quantitatively determined according to the method suggested by SALKOWSKI: Shaking out the oxalic acid from the acidified urine by means of ether. The method suggested by AUTENRIETH and BARTH is as follows:

The twenty-four-hour urine is precipitated by $CaCl_2$ and ammonia in excess. After 18–20 hours the precipitate is collected (the filtrate must be clear) and dissolved in a little hydrochloric acid and shaken out 4–5 times with 150–200 cc. ether (containing 3 per cent absolute alcohol). The united ethereal extracts are filtered through a dry filter and distilled after the addition of about 5 cc. of water. The liquid, if necessary, is decolorized with animal charcoal and precipitated with $CaCl_2$ and ammonia, made acid after a certain time with acetic acid, and finally the oxalate is collected, washed, burned to CaO, and weighed. MACLEAN[2] finds that this method yields too low results, as some calcium oxalate always remains in the filtrate and the original method as suggested by SALKOWSKI is more trustworthy.

Allantoin (GLYOXYLDIUREIDE), $C_4H_6N_4O_3$, $OC\begin{cases} NH.CH.HN.CO.NH_2, \\ \quad\quad | \\ NH.CO \end{cases}$

occurs in the urine of children within the first eight days after birth, and in very small amounts also in the urine of adults (GUSSEROW, ZIEGLER and HERMANN). It is found in rather abundant quantities in the urine of pregnant women (GUSSEROW). According to WIECHOWSKI the urine of adults, if it contains any allantoin at all, has only traces, and he could not detect any in the urine of nurslings or in the amniotic fluid, which does not agree with previous reports. Allantoin has also been found in the urine of suckling calves (WÖHLER), in urine of oxen (SALKOWSKI), and sometimes in the urine of other animals (MEISSNER). WIECHOWSKI has found it in relatively large quantities in the urine of the dog, cat, rabbit and monkey, and he considers that allantoin is a terminal metabolic product in these animals. It is also found, as first shown by VAUQUELIN and LASSAIGNE,[3] in the allantoic fluid of the cow (hence the name). That allantoin is formed from the uric acid in mammalia but not in human beings (WIECHOWSKI) is almost certain, and the investigations on which this is based have already been given in discussing the decomposition of uric acid. The allantoin thus originates from the purine bodies, and

[1] See Wiener, Ergebnisse der Physiol., **1**, Abt. 1.

[2] Salkowski, Zeitschr. f. physiol. Chem., **29**; Autenrieth and Barth, *ibid.*, **35**; MacLean, *ibid.*, **60**.

[3] Ziegler and Hermann, see Gusserow, Arch. f. Gynäkol, **3**—both cited from Huppert-Neubauer, Harn-Analyse, 10. Aufl., 377; Wöhler, Annal. d. Chem. u. Pharm., **70**; Salkowski, Zeitschr. f. physiol. Chem., **42**; Meissner, Zeitschr. f. rat. Med. (3), **31**; Lassaigne, Annal. de Chim. et Phys., **17**; Wiechowski, Hofmeister's Beiträge, **11**, and Arch. f. exp. Path. u. Pharm., **60**.

consequently the excretion of allantoin is considerably increased, according to MINKOWSKI, COHN, SALKOWSKI, and MENDEL and BROWN,[1] after feeding thymus or pancreas. A strong allantoin excretion is also found in dogs after poisoning with hydrazine (BORISSOW), hydroxylamine, semicarbazide, and aminoguanidine (POHL), and this increase in the excretion of allantoin is connected with the nuclein metabolism. POHL[2] has found in dogs on poisoning with hydrazine that the liver contained allantoin and that other organs contained traces, while it does not exist in the organs of normal dogs, and he has also detected the formation of allantoin in the autolysis of the intestinal mucosa, liver, thymus, spleen and pancreas. It is very probable that in these cases we are dealing with a destruction of cells and an enzymoytic uric acid formation with a subsequent uricolysis with the formation of allantoin. According to PODUSCHKA and MINKOWSKI,[3] allantoin introduced into dogs appears almost entirely in the urine, while in man only a small portion of the ingested substance is eliminated in the urine and seems in the last case to be chiefly burnt.

Allantoin is a colorless substance often crystallizing in prisms, difficultly soluble in cold water, easily soluble in boiling water, and also in warm alcohol, but not soluble in cold alcohol or ether. A watery allantoin solution gives no precipitate with silver nitrate alone, but by the careful addition of ammonia a white flocculent precipitate is formed, $C_4H_5AgN_4O_3$, which is soluble in an excess of ammonia and which consists after a certain time of very small, transparent microscopic globules. The dry precipitate contains 40.75 per cent silver. A watery allantoin solution is precipitated by mercuric nitrate. On continued boiling allantoin reduces FEHLING'S solution. It gives SCHIFF'S furfurol reaction less rapidly and less intensely than urea. Allantoin does not give the murexide test.

Allantoin is most easily prepared by the oxidation of uric acid with lead peroxide. In preparing allantoin from urine, proceed according to LOEWI'S method, which consists of the following: The faintly acidified urine is precipitated with a mercurous-nitrate solution, the filtrate treated with H_2S, and the new filtrate precipitated by magnesium oxide and silver nitrate after the removal of the H_2S. The precipitate is filtered off and washed with warm water and decomposed with H_2S, and the

[1] Minkowski, Arch. f. exp. Path. u. Pharm., **41**, and Centralbl. f. innere Med., 1898; Cohn, Zeitschr. f. physiol. Chem., **25**; Salkowski, Centralbl. f. d. med. Wissensch., 1898; Mendel and Brown, Amer. Journ. of Physiol., **3**.

[2] Borissow, Zeitschr. f. physiol. Chem., **19**; Pohl, Arch. f. exp. Path. u. Pharm., **46**; Poduschka, *ibid.*, **44**. According to Underhill and Kleiner, Journ. of biol. Chem., **4**, hydrazine has no other action on the excretion of allantoin than that caused by the refusal to take food brought about by the poison.

[3] Poduschka, Arch. f. exp. Path. u. Pharm., **44**; Minkowski, *ibid.*, **41**.

filtrate evaporated to dryness. The residue is extracted with hot water and then the solution is precipitated with mercuric nitrate. The precipitate is collected and decomposed by H_2S. From the evaporated filtrate the allantoin crystallizes out. This method, DAKIN claims, is not suited for the quantitative estimation of allantoin. For this purpose we make use of the method suggested by WIECHOWSKI,[1] which consists in precipitating the allantoin by a dilute mercuric acetate solution in the presence of concentrated sodium acetate solution. For details we refer to the original publications.

Glyoxylic Acid, $C_2H_4O_4$, $\overset{CH(OH)_2}{\underset{COOH}{\cdot}}$, is produced on boiling allantoin as well as uric acid with alkalies and also on the oxidation of many substances, among which we can mention creatine and creatinine. It is also of interest that allantoin can be prepared synthetically from glyoxylic acid and urea and that glyoxylic acid yields oxalic acid when introduced into the body. The reports in regard to its occurrence in the urine conflict,[2] as it is readily destroyed in the body, and its passage into the urine is very improbable, or at least only occurs seldom.

Hippuric Acid (BENZOYL-AMINO ACETIC ACID), $C_9H_9NO_3$, $\overset{OC.C_3H_5}{\underset{HN.CH_2.COOH}{\cdot}}$.

This acid decomposes into benzoic acid and glycocoll on boiling with mineral acids or alkalies, and also in the putrefaction of the urine. The reverse of this occurs if these two components are heated in a sealed tube, according to the following equation: $C_6H_5COOH + NH_2.CH_2.COOH = C_6H_5.CO.NH.CH_2.COOH + H_2O$. This acid may be synthetically prepared from benzamide and monochloracetic acid, $C_6H_5.CO.NH_2 + CH_2Cl.COOH = C_6H_5.CO.NH.CH_2.COOH + HCl$, and in various other ways, but most simply from glycocoll and benzoyl chloride in the presence of alkali.

Hippuric acid occurs in large amounts in the urine of herbivora, but only in small quantities in that of carnivora. The quantity of hippuric acid eliminated in human urine on a mixed diet is usually less than 1 gram per day; as an average it is 0.7 gram. After eating freely of vegetables and fruit, especially such fruit as plums, the quantity may be more than 2 grams. Hippuric acid is also found in the perspiration, the blood, the suprarenal capsule of oxen, and in ichthyosis scales. Nothing is positively known in regard to the quantity of hippuric acid in the urine in disease.

The Formation of Hippuric Acid in the Organism. Benzoic acid and also the substituted benzoic acids are converted into hippuric acid and substituted hippuric acids within the body. Moreover, those bodies are transformed into hippuric acid which by oxidation (toluene, cinnamic

[1] Loewi, *ibid.*, **44**; Wiechowski, Hofmeister's Beiträge, **11**, and Arch. f. exp. Path. u. Pharm., **60**; Dakin, Journ. of biol. Chem., **3**, 73.

[2] The literature on the occurrence and detection of glyoxylic acid in the urine can be found in Granström, Hofmeister's Beiträge, **11**.

acid, hydrocinnamic acid) or by reduction (quinic acid) are converted into benzoic acid. The question of the origin of hippuric acid is therefore connected with the question of the origin of benzoic acid; the formation of the second component, glycocoll, from the protein substances in the body is unquestionable.

Hippuric acid is found in the urine of starving dogs (SALKOWSKI), also in dog's urine after a diet consisting entirely of meat (MEISSNER and SHEPHERD, SALKOWSKI, and others [1]). It is evident that the benzoic acid originates in these cases from the proteins, and it is generally admitted that it is produced by the putrefaction of proteins in the intestine. Among the products of the putrefaction of protein outside of the body SALKOWSKI found phenylpropionic acid, $C_6H_5.CH_2.CH_2.COOH$, which is oxidized in the organism to benzoic acid and eliminated as hippuric acid after combining with glycocoll. Phenylpropionic acid seems to be formed from the aminophenylpropionic acid (phenylalanine), which is derived from several protein substances. The supposition that the phenylpropionic acid is produced from tyrosine by putrefaction of the intestine has not been substantiated by the researches of BAUMANN, SCHOTTEN, and BAAS.[2] The importance of putrefaction in the intestine in producing hippuric acid is evident from the fact that after thoroughly disinfecting the intestine of dogs with calomel the hippuric acid disappears from the urine (BAUMANN [3]).

The large quantity of hippuric acid present in the urine of herbivora is partly explained by the specially active processes of putrefaction going on in the intestine of these animals. According to VASILIU [4] this can hardly be correct, because, as he has found by feeding sheep with casein, this would require a too intense putrefaction of the protein (indeed 40 per cent of it). This author's explanation lies in part that in the herbivora only a small part of the phenylalanine is burnt, and is used to a greater extent in the formation of hippuric acid than in man and carnivora, and in part by the fact that the food of herbivora contains larger quantities of a non-nitrogenous mother substance of the benzoic acid. There is hardly any doubt that the hippuric acid in human urine after a mixed diet, and especially after a diet of vegetables and fruits, originates in part from the aromatic substances, e.g., quinic acid.

The view proposed by WEISS and others that a parallelism exists between the excretion of hippuric acid and uric acid in that an increase in the first is

[1] Salkowski, Ber. d. deutsch. chem. Gesellsch., **11**; Meissner and Shepard, Untersuch. über das Entstehen der Hippursäure im thierischen Organismus. Hanover, 1866.

[2] E. and H. Salkowski, Ber. d. deutsch. chem. Gesellsch., **12**; Baumann, Zeitschr. f. physiol. Chem., **7**; Schotten, *ibid.*, 8; Baas, *ibid.*, **11**.

[3] Ibid., **10**, 131.

[4] Vasiliu, Mitt. d. landwirt. Inst. Breslau, Bd. 4, 1907.

followed by a diminution in the second, and that, for example, quinic acid produces a diminution in the excretion of uric acid corresponding to the increased formation of hippuric acid (WEISS, LEWIN), cannot be considered as sufficiently proven (HUPFER[1]).

As the thorough investigations of WIECHOWSKI teach, the synthesis of hippuric acid does not stand in any direct relation to the extent of protein metabolism; it varies, on the contrary, with the duration of circulation of benzoic acid and the quantity of glycocoll present in the body. The amount of the latter in intermediary metabolism is so great that in rabbits, on the administration of benzoic acid, more than one-half of the total urine nitrogen may exist as glycocoll. MAGNUS-LEVY[2] found in rabbits and sheep up to 27.8 per cent of the total nitrogen as hippuric-acid nitrogen, and both investigators have found so much hippuric-acid nitrogen that it could not be accounted for by the glycocoll preformed from the proteins, which amounts to about 4–5 per cent of the total nitrogen of the protein of the food and body.

In carnivora (dog) and man the conditions are different, according to BRUGSCH and R. HIRSCH, FEIGEN and BRUGSCH, as here there is no more glycocoll available for hippuric acid formation than is split off from the proteins on hydrolysis. LEWINSKI[3] believes that, nevertheless, in man after abundance of benzoic acid about 34 per cent of the total nitrogen may be excreted as hippuric acid, but BRUGSCH claims that these observations are incorrect. The abundant production of hippuric acid in herbivora induced ABDERHALDEN, GIGON and STRAUSS to investigate the comparative supply of certain amino-acids in carnivora and herbivora, and they found in cats, rabbits and hens that the percentage quantity of glycocoll split off from the entire organism (with the exception of the intestinal contents and fat and feathers) by hydrolysis was the same, namely 2.33 to 3.34 per cent of the proteins. In order to account for the large quantity of glycocoll which can be eliminated as hippuric acid, we must admit of a formation of glycocoll from complexes rich in carbon, and it is quite possible that the benzoic acid combines with higher amino-acids and that the hippuric acid is then formed from this combination by oxidation. The investigations of MAGNUS-LEVY[4] to prove this

[1] Weiss, Zeitschr. f. physiol. Chem., **25**, **27**, **38**; Lewin, Zeitschr. f. klin. Med., **42**; Hupfer, Zeitschr. f. physiol. Chem., **37**. See also Wiener, "Die Harnsäure," Ergebnisse der Physiol., **1**, Abt. 1.

[2] Wiechowski, Hofmeister's Beiträge, **7** (literature); A. Magnus-Levy, Münch. med. Wochenschr., 1905.

[3] Brugsch and Hirsch, Zeitschr. f. exp. Path. u. Therap., **3**; Brugsch, Maly's Jahresber., **37**, 621, and Bioch. Centralbl., 8, 336; Feigen, Maly's Jahresber., **36**, 631; Lewinski, Arch. f. exp. Path. u. Pharm., **58**.

[4] Abderhalden, Gigon and Strauss, Zeitschr. f. physiol. Chem., **51**; Magnus-Levy, Bioch. Zeitschr., **6**.

assumption, where he used benzoylated higher amino-acids, have not given support to this assumption, and the question how the abundant formation and elimination of glycocoll take place is still unexplained.

The kidneys may be considered in dogs as special organs for the synthesis of hippuric acid (SCHMIEDEBERG and BUNGE [1]). In other animals, as in rabbits, the formation of hippuric acid seems to take place in other organs, such as the liver and muscles. The synthesis of hippuric acid is therefore not exclusively limited to any special organ, though perhaps in some species of animals it may be more abundant in one organ than in another.

Properties and Reactions of Hippuric Acid. This acid crystallizes in semi-transparent, long, four-sided, milk-white, rhombic prisms or columns, or in needles by rapid crystallization. They dissolve in 600 parts cold water, but more easily in hot water. They are easily soluble in alcohol, but with difficulty in ether. The acid dissolves more easily (about 12 times) in acetic ether than in ethyl ether. Petroleum-ether does not dissolve hippuric acid.

On heating hippuric acid it first melts at 187.5° C. to an oily liquid which crystallizes on cooling. On continued heating it decomposes, producing a red mass and a sublimate of benzoic acid, with the generation, first, of a peculiar pleasant odor of hay and then an odor of hydrocyanic acid. Hippuric acid is easily differentiated from benzoic acid by this behavior, also by its crystalline form and its insolubility in petroleum ether. Hippuric acid and benzoic acid both give LÜCKE'S reaction, namely, they generate an intense odor of nitrobenzene when evaporated to dryness with nitric acid and when the residue is heated with sand in a glass tube. Hippuric acid in most cases forms crystallizable salts, with bases. The combinations with alkalies and alkaline earths are soluble in water and alcohol. The silver, copper, and lead salts are soluble with difficulty in water; the ferric salt is insoluble.

Hippuric acid is best prepared from the fresh urine of a horse or cow. The urine is boiled a few minutes with an excess of milk of lime. The liquid is filtered while hot, concentrated and then cooled, and the hippuric acid precipitated by the addition of an excess of hydrochloric acid. The crystals are pressed, dissolved in milk of lime by boiling, and treated as above; the hippuric acid is precipitated again from the concentrated filtrate by hydrochloric acid. The crystals are purified by recrystallization and decolorized, when necessary, by animal charcoal.

The quantitative estimation of hippuric acid in the urine may be performed by the following method (BUNGE and SCHMIEDEBERG [2]):

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[1] Arch. f. exp. Path. u. Pharm., **6**; also A. Hoffmann, *ibid.*, **7**, and Kochs, Pflüger's Arch., **20**; Bashford and Cramer, Zeitschr. f. physiol. Chem., **35**.

[2] Arch. f. exp. Path. u. Pharm., **6**. In regard to other methods, such as Blumen-

The urine is first made faintly alkaline with soda, evaporated nearly to dryness, and the residue thoroughly extracted with strong alcohol. After the evaporation of the alcohol the residue is dissolved in water, the solution acidified with sulphuric acid, and completely extracted by agitating (at least five times) with fresh portions of acetic ether. The acetic ether is then repeatedly washed with water, which is removed by means of a separatory funnel, then evaporated at a medium temperature and the dry residue treated repeatedly with petroleum-ether, which dissolves the benzoic acid, oxyacids, fats, and phenols, while the hippuric acid remains undissolved. This residue is now dissolved in a little warm water and evaporated at 50–60° C. to crystallization. The crystals are collected on a small weighed filter. The mother-liquor is repeatedly shaken with acetic ether. This last is removed and evaporated; the residue is added to the above crystals on the filter, dried and weighed.

Phenaceturic Acid, $C_{10}H_{11}NO_3 = C_6H_5.CH_2.CO.NH.CH_2.COOH$. This acid, which is produced in the animal body by a combination of glycocoll with the phenyl-acetic acid, $C_6H_5.CH_2.COOH$, formed in the putrefaction of the proteins, has been prepared from horse's urine by Salkowski,[1] but it probably also occurs in human urine. According to Vasiliu [2] it is just as important a constituent of the urine of herbivora as hippuric acid is.

Benzoic Acid, $C_7H_6O_2$ or $C_6H_5.COOH$, is found in rabbit's urine and sometimes, though in small amounts, in dog's urine (Weyl and v. Anrep). According to Jaarsveld and Stokvis and to Kronecker it is also found in human urine in diseases of the kidneys. The occurrence of benzoic acid in the urine seems to be due to a fermentative decomposition of hippuric acid. Such a decomposition may very easily occur in an alkaline urine or in one containing proteid (Van de Velde and Stokvis). In certain animals—pigs and dogs—the kidneys, according to Schmiedeberg and Minkowski,[3] contain a special enzyme, Schmiedeberg's *histozym*, which splits the hippuric acid with the separation of benzoic acid.

Ethereal Sulphuric Acids. In the putrefaction of proteins in the intestine, phenols,—whose mother-substance is considered to be tyrosine,—indol and skatol are produced. These phenols directly, and the two last-named bodies after they have been oxidized respectively into indoxyl and skatoxyl, pass into the urine as ethereal sulphuric acids after uniting with sulphuric acid. The most important of these ethereal acids are *phenol-* and *cresol-sulphuric acids*—which were formerly also called phenol-forming substances—*indoxyl-* and *skatoxyl-sulphuric acids*. To this group also belong *pyrocatechin-sulphuric acid*, which occurs only in very small amounts in human urine, and *hydroquinone-sulphuric acid*, which appears in the urine after poisoning with phenol, and under physiological conditions perhaps other ethereal acids occur which have

thal as well as Pfeiffer, Bloch and Riecke, see Maly's Jahresber., **30** and **32**. See also Wiechowski, l. c.

[1] Zeitschr. f. physiol. Chem., **9**.

[2] Mitteil. d. landw. Inst., Breslau, **4**.

[3] Weyl and v. Anrep. Zeitschr. f. physiol. Chem., **4**; Jaarsveld and Stokvis, Arch. f. exp. Path. u. Pharm., **10**; Kronecker, *ibid.*, **16**; Van de Velde and Stokvis, *ibid.*, **17**; Schmiedeberg, *ibid.*, **14**, 379; Minkowski, *ibid.*, **17**.

not been isolated. The ethereal sulphuric acids of the urine were discovered and specially studied by BAUMANN.[1] The quantity of these acids in human urine is small, while horse's urine contains larger quantities. According to the determinations of v. D. VELDEN the quantity of ethereal sulphuric acid in human urine in twenty-four hours varies between 0.094 and 0.620 gram. The relation of the sulphate-sulphuric acid *A* to the conjugated sulphuric acid *B*, in health, is on an average 10:1. It undergoes such great variations, as found by BAUMANN and HERTER,[2] and after them by many other investigators, that it is hardly possible to consider the average figures as normal. After taking phenol and certain other aromatic substances, as well as when putrefaction within the organism is general, the elimination of ethereal sulphuric acid is greatly increased. On the contrary, it is diminished when the putrefaction in the intestine is reduced or prevented. For this reason it may be greatly diminished by carbohydrates and exclusive milk diet.[3] The intestinal putrefaction and the elimination of ethereal sulphuric acid have also been diminished in some cases by certain therapeutic agents which have an antiseptic action; still the investigators do not agree in their reports.[4]

Great importance has been given to the relation between the total sulphuric acid and the conjugated sulphuric acid, or between the conjugated sulphuric acid and the sulphate-sulphuric acid, in the study of the intensity of the putrefaction in the intestine under different conditions. Several investigators, F. MÜLLER, SALKOWSKI, and v. NOORDEN,[5] consider correctly that this relation is only of secondary value, and that it is more correct to consider the absolute value. It must be remarked that the absolute values for the conjugated sulphuric acid also undergo great variation, so that it is at present impossible to give the upper or lower limit for the normal value.

Phenol- and p-Cresol-sulphuric Acids, $C_6H_5.O.SO_2.OH$ and $C_6H_4\begin{<}\begin{matrix}O.SO_2.OH\\CH_3\end{matrix}$. These acids are found as alkali salts in human urine, in which also orthocresol has been detected. The quantity of cresol-

[1] Pflüger's Arch., **12** and **13**.

[2] v. d. Velden, Virchow's Arch., **70**; Herter, Zeitschr. f. physiol. Chem., **1**.

[3] See Hirschler, Zeitschr. f. physiol. Chem., **10**; Biernacki, Deutsch. Arch. f. klin. Med., **49**; Rovighi, Zeitschr. f. physiol. Chem., **16**; Winternitz, *ibid.*, and Schmitz, *ibid.*, **17** and **19**.

[4] See Baumann and Morax, Zeitschr. f. physiol. Chem., **10**; Steiff, Zeitschr. f. klin. Med., **16**; Rovighi, l. c., Stern, Zeitschr. f. Hyg., **12**; and Bartoschewitsch, Zeitschr. f. physiol. Chem., **17**; Mosse, *ibid.*, **23**.

[5] Müller, Zeitschr. f. klin. Med., **12**; v. Noorden, *ibid.*, **17**; Salkowski, Zeitschr. f. physiol. Chem., **12**.

sulphuric acid is considerably greater than of phenol-sulphuric acid. In the quantitative estimation the phenols are set free from the two ethereal acids and determined together as tribromphenol. The quantity of phenols which are separated from the ethereal-sulphuric acids of the urine amounts to 17–51 milligrams in the twenty-four hours (MUNK). The methods for the quantitative estimation used heretofore give, according to RUMPF, as well as KOSSLER and PENNY,[1] such inaccurate results that new determinations are very desirable. After a vegetable diet the quantity of these ethereal-sulphuric acids is greater than after a mixed diet. After the ingestion of carbolic acid, which is in great part converted by synthesis within the organism into phenol-sulphuric acid, also into pyrocatechin- and hydroquinon-sulphuric acid,[2] or when the amount of sulphuric acid is not sufficient to combine with the phenol, it forms phenyl-glucuronic acid,[3] the quantity of phenols and ethereal-sulphuric acids in the urine is considerably increased at the expense of the sulphate-sulphuric acid. The same is also true of other phenols.

An increased elimination of phenol-sulphuric acids occurs in active putrefaction in the intestine with stoppage of the contents of the intestine, as in ileus, diffused peritonitis with atony of the intestine, or tuberculous enteritis, but not in simple obstruction. The elimination is also increased by the absorption of the products of putrefaction from purulent wounds or abscesses. An increased elimination of phenol has been observed in a few other cases of diseased conditions of the body.[4]

The alkali salts of phenol- and cresol-sulphuric acids crystallize in white plates, similar to mother-of-pearl, which are rather freely soluble in water. They are soluble in boiling alcohol, but only slightly soluble in cold alcohol. On boiling with dilute mineral acids they are decomposed into sulphuric acid and the corresponding phenol.

Phenol-sulphuric acids have been synthetically prepared by BAUMANN from potassium pyrosulphate and potassium phenolate or *p*-cresolate. For the method of their preparation from urine, which is rather complicated, and also for the known phenol reactions, the reader is referred to other text-books. The quantitative estimation of these ethereal-sulphuric acids was usually made by weighing the phenol which was separated from the urine as tribromphenol. At the present time the following method is employed:

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[1] Munk, Pflüger's Arch., **12**; Rumpf, Zeitschr. f. physiol. Chem., **16**; Kossler and Penny, *ibid.*, **17**.

[2] See Baumann, Pflüger's Arch., **12** and **13**, and Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156.

[3] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **14**.

[4] See G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **12** (this contains also all references to the literature on this subject); Fedeli, Moleschott's Untersuch., **15**.

Kossler and Penny's Method with Neuberg's[1] modification. The liquid containing phenol is treated with N/10 caustic soda until strongly alkaline, warmed on the water-bath in a flask with a glass stopper, and then treated with an excess of N/10 iodine solution, the quantity being exactly measured. Sodium iodide is first formed and then sodium hypoiodite, which latter forms tri-iodophenol with the phenol according to the following equation:

$$C_6H_5OH + 3NaIO = C_6H_2I_3.OH + 3NaOH.$$

On cooling, acidify with sulphuric acid and determine the excess of iodine by titration with N/10 sodium thiosulphate solution. This process is also available for the estimation of paracresol. Each cubic centimeter of the iodine solution used is equivalent to 1.5670 milligrams of phenol or 1.8018 milligrams of cresol. As the determination does not give any idea as to the variable proportions of the two phenols, the quantity of iodine used must be calculated as one or the other of the two phenols. Before such a determination is carried out, the concentrated urine is first distilled after acidification with sulphuric acid and the distillate purified by precipitation with lead, and distilled again (Neuberg). For details, see Neuberg, l. c., and Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

The methods for the separate determination of the conjugated sulphuric acid and the sulphate-sulphuric acid will be spoken of later in connection with the determination of the sulphuric acid of the urine.

Pyrocatechin-sulphuric Acid. This acid was first found in horse's urine in rather large quantities by Baumann. It occurs in human urine only in the very smallest amounts, and perhaps not constantly, but it is present abundantly in the urine after taking phenol, pyrocatechin, or protocatechuic acid.

With an exclusively meat diet this acid does not occur in the urine, and it therefore must originate from vegetable food. It probably originates from the protocatechuic acid, which, according to Preusse, passes in part into the urine as pyrocatechin-sulphuric acid. This acid may also perhaps be formed by the oxidation of phenol within the organism (Baumann and Preusse[2]).

Pyrocatechin, or o-Dioxybenzene, $C_6H_4(OH)_2$, was first observed in the urine of a child (Ebstein and J. Müller). The reducing body alcapton, first found by Bödeker[3] in human urine and which was considered for a long time as identical with pyrocatechin, is in most cases probably *homogentisic acid* (see below).

Pyrocatechin crystallizes in prisms which are soluble in alcohol, ether, and water. It melts at 102–104° C., and sublimes in shining plates. The watery solution becomes green, brown, and ultimately black in the presence of alkali and the oxgyen of the air. If very dilute ferric chloride is treated with tartaric acid and then made alkaline with ammonia, and this added to a watery solution of pyrocatechin, we obtain a violet or cherry-red liquid which becomes green on adding excess of acetic acid. Pyrocatechin is precipitated by lead acteate. It reduces an ammoniacal silver solution at the ordinary temperature, and with heat reduces alkaline copper-oxide solutions but does not reduce bismuth oxide.

A urine containing pyrocatechin, if exposed to the air, especially when alkaline, quickly becomes dark and reduces alkaline copper solutions when heated. In

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[1] Kossler and Penny, Zeitschr. f. physiol. Chem., 17; Neuberg, *ibid.*, 27.

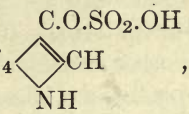
[2] Baumann and Herter, Zeitschr. f. physiol. Chem., 1; Preusse, *ibid.*, 2; Baumann, *ibid.*, 3.

[3] Ebstein and Müller, Virchow's Arch., 62; Bödeker, Zeitschr. f. rat. Med. (3), 7.

detecting pyrocatechin in the urine it is concentrated when necessary, filtered, boiled with the addition of sulphuric acid to remove the phenols, and repeatedly shaken, after cooling, with ether. The ether is distilled from the several ethereal extracts, the residue neutralized with barium carbonate and shaken again with ether. The pyrocatechin which remains after evaporating the ether may be purified by recrystallization from benzene.

Hydroquinone, or P-DIOXYBENZENE, $C_6H_4(OH)_2$, often occurs in the urine after the use of phenol (BAUMANN and PREUSSE). The dark color which certain urines, so-called "carbolic urines," assume in the air is due to decomposition products. Hydroquinone does not occur as a normal constituent of urine, but only after the administration of hydroquinone; and according to LEWIN,[1] it may be found in the urine of rabbits as an ethereal-sulphuric acid, being a decomposition product of arbutin.

Hydroquinone forms rhombic crystals which are readily soluble in water, alcohol, and ether. It melts at 169° C. Like pyrocatechin, it easily reduces metallic oxides. It acts like pyrocatechin with alkalies, but is not precipitated by lead acetate. It is oxidized into quinone by ferric chloride and other oxidizing agents, and quinone can be detected by its peculiar odor. Hydroquinone-sulphuric acid is detected in the urine by the same methods as pyrocatechin-sulphuric acid.

Indoxyl-sulphuric Acid, $C_8H_7NSO_4$, 

, also called URINE INDICAN, formerly called UROXANTHINE (HELLER), occurs as an alkali-salt in the urine. This acid is the mother-substance of a great part of the indigo of the urine. The quantity of indigo which can be separated from the urine is considered as a measure of the quantity of indoxyl-sulphuric acid (and indoxyl-glucuronic acid) contained in the urine. This amount, according to JAFFÉ,[2] for man is 5–20 milligrams per twenty-four hours. Horse's urine contains about twenty-five times as much indigo-forming substance as human urine.

Indoxyl-sulphuric acid is derived, as previously mentioned (page 492), from indol, which is first oxidized in the body into indoxyl and is then conjugated with sulphuric acid. After subcutaneous injection of indol the elimination of indican is considerably increased (JAFFÉ, BAUMANN and BRIEGER, and others). It is also increased by the introduction in the animal organism of orthonitrophenolpropiolic acid (G. HOPPE-SEYLER[3]). Indol is formed by the putrefaction of proteins. The putrefaction of secretions rich in protein in the intestine also explains the occurrence of indican in the urine during starvation. Gelatin, on the contrary, does not increase the elimination of indican.

An abnormally increased elimination of indican occurs in those

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[1] Virchow's Arch., **92**.

[2] Pflüger's Arch., **3**.

[3] Jaffé, Centralbl. f. d. med. Wissensch., 1872; Baumann and Brieger, Zeitschr. f. physiol. Chem., **3**; G. Hoppe-Seyler, *ibid.*, **7** and **8**. See also Porcher and Hervieux, Journ. de Physiol., **7**.

diseases where the small intestines are obstructed, causing an increased putrefaction and thus producing an abundance of indol. Such an increased elimination of indican occurs on tying the small intestine of a dog, but not the large intestine (JAFFÉ), an observation which has been recently confirmed by ELLINGER and PRUTZ.[1] They removed an intestine loop in dogs and replaced it in a reversed position, the distal end of the loop being attached to the proximal end of the intestine, and in this manner, by the inverted peristalsis so obtained, they effected a disturbance in the movement of the intestinal contents. It was shown that this obstruction in the small intestine caused an increased elimination of indican, while an obstruction in the large intestine showed no such action.

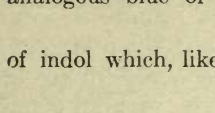
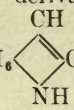
The putrefaction of proteins in other organs and tissues besides the intestine may also cause an increase in the indican of the urine. Certain investigators, BLUMENTHAL, ROSENFELD, and LEWIN, claim to have shown that an increased excretion of indican can also be brought about without putrefaction by an increased destruction of tissue in starvation and also after phlorhizin poisoning; but these statements are vehemently opposed by other investigators, such as P. MAYER, SCHOLZ, and ELLINGER, and are improbable. The indol, it seems, is not formed from the tryptophane (indolaminopropionic acid) as intermediary step in the demolition of the proteins in the animal body, but rather from the putrefaction of the tryptophane in the intestine. GENTZEN,[2] has also shown that tryptophane introduced subcutaneously or per os into the body does not lead to an indicanuria, but only when it is exposed to bacterial decomposition in the large intestine. The reports as to the elimination of indican after oxalic-acid poisoning are conflicting. After poisoning with oxalic acid HARNACK and v. LEYEN found an increased indican elimination, and MORACZEWSKI believes he has proven a certain parallelism between the quantity of indican and the quantity of oxalic acid in diabetes. SCHOLZ,[3] on the contrary, obtained no increase in the excretion of indican after oxalic-acid poisoning.

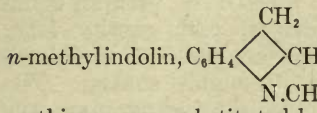
The excretion of indican is, as above stated, increased by the introduction of indol, but also by indoxyl or indoxyl-carboxylic acid. Indol-carboxylic acid, on the contrary, does not yield indican, but, according to PORCHER and HERVIEUX, another chromogen. BENEDICENTI has also shown that indigo blue or

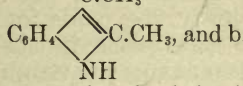
[1] Jaffé, Virchow's Arch., **70**; Ellinger and Prutz, Zeitschr. f. physiol. Chem., **38**.

[2] Blumenthal, Arch. f. (Anat. u.) Physiol., 1901, Suppl., and 1902, with Rosenfeld, Charité annalen, **27**, and Hofmeister's Beiträge, **5**; Lewin, Hofmeister's Beiträge, **1**; Mayer, Arch. f. (Anat. u.) Physiol., 1902, Zeitschr. f. klin. Med., **47**, and Zeitschr. f. physiol. Chem., **29**, **32**; Scholz, *ibid.*, **38**; Ellinger, *ibid.*, **39**; Gentzen, "Ueber die Vorstufen des Indols bei der Eiweissfäulnis im Thierkörper," Inaug.-Dissert. Königsberg, 1904.

[3] Harnack, Zeitschr. f. physiol. chemie, **29**; Scholz, l. c., Moraczewski, Centralbl. f. innere Med., 1903.

analogous blue or green pigments are produced only from those derivatives of indol which, like *n*-methyl indol   or



methine groups substituted by alkyl. From those derivatives in which one or two hydrogen atoms are substituted by alkyl, such as α-methyl indol, dimethyl indol,  are produced, a behavior which PORCHER and HERVIEUX [1] have observed in several alkyl-substituted indols.

An increased elimination of indican has been observed in many diseases,[2] and in these cases the quantity of phenol eliminated is also generally increased. A urine rich in phenol is not always rich in indican.

The potassium salt of indoxyl-sulphuric acid, which was prepared pure by BAUMANN and BRIEGER from the urine of a dog fed on indol, has subsequently been prepared synthetically by BAUMANN and THESEN,[3] by fusing phenyl-glycine-orthocarboxylic acid with alkali and then from this producing the indoxylsulphate by means of potassium pyrosulphate. It crystallizes in colorless, shining plates or leaves which are easily soluble in water, but less readily in alcohol. It is split by mineral acids into sulphuric acid and indoxyl. The latter without access of air passes into a red compound, indoxyl red, but in the presence of oxidizing reagents is converted into indigo blue: $2C_8H_7NO+2O=C_{16}H_{10}N_2O_2+2H_2O$. The detection of indican is based on this last fact.

For the rather complicated preparation of indoxyl-sulphuric acid as potassium salt from urine the reader is referred to other text-books. For the detection of indican in urine in ordinary cases the following method of JAFFÉ-OBERMAYER, which also serves as an approximate test for the quantity of indican, is sufficient.

JAFFÉ-OBERMAYER'S *Indican Test.* JAFFÉ uses chloride of lime as the oxidizing agent, while OBERMAYER employs ferric chloride. Other oxidizing agents have been suggested, such as potassium permanganate, potassium dichromate, alkali chlorate, and hydrogen peroxide (the

[1] The work of Porcher and Hervieux can be found in Compt. rend., **145**, Compt. rend. soc. biol., **62**, and Bull. soc. chim. (4), **1**; Benedicenti, Zeitschr. f. physiol. Chem., **53** and Arch. f. exp. Path. u. Pharm., 1908, Suppl. (Schmiedeberg's Festschr.).

[2] See Jaffé, Pflüger's Arch., **3**; Senator, Centralbl. f. d. med. Wissensch., 1877; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **12** (contains older literature); also Berl. klin. Wochenschr., 1892.

[3] Baumann with Brieger, Zeitschr. f. physiol. Chem., **3**; with Thesen, *ibid.*, **23**.

latter suggested by PORCHER and HERVIEUX [1]). With OBERMAYER'S reagent the test is performed as follows:

The acid urine (if alkaline it must be acidified with acetic acid) (ELLINGER) is precipitated with basic lead acetate, 1 cc. for every 10 cc. of the urine. 20 cc. of the filtrate are treated in a test-tube with an equal volume of pure concentrated hydrochloric acid (specific gravity 1.19) which contains 2–4 grams ferric chloride to the liter, and 2–3 cc. chloroform are added and the mixture immediately thoroughly shaken. The chloroform is thereby colored more or less blue, depending upon the amount of indican. Besides indigo blue we may also have indigo red produced, whose formation has been explained in various ways. The quantity of indigo red becomes greater the more slowly the oxidation takes place, and especially when the decomposition takes place in the warmth (see the works of ROSIN, BOUMA, WANG, MAILLARD, ELLINGER and HERVIEUX [2]).

According to ELLINGER the source of the indigo-red formation may be the isatin that is produced by the superoxidation of the indoxyl by the action of the reagent, and this isatin forms indigo red with the indoxyl in the hydrochloric-acid solution. MAILLARD, on the contrary, is of the view that the blue substance which is taken up by the chloroform from the urine mixed with hydrochloric acid is not indigotin (indigo-blue), but another substance, called by him hemi-indigotin, which in alkaline solution polymerizes immediately into indigotin, while in acid reaction it is converted into indirubin (indigo red).

The chloroform solution of indigo obtained in the indican test may be used in the quantitative colorimetric determination by comparison with a solution of indigo in chloroform of known strength (KRAUSS and ADRIAN). WANG and others convert the indigo into indigo-sulphonic acid by concentrated sulphuric acid and titrate with potassium permanganate. There is still doubt as to the surest and most trustworthy method for the determination of indican, and especially as to the question how the indigo residue is to be washed (see WANG, BOUMA, ELLINGER, and SALKOWSKI [3]), and for this reason we shall refer only to the works cited above.

Because of the difficulty arising from the production of indirubin in addition to indigotin, BOUMA has recommended the conversion of all the indoxyl into indirubin by boiling the urine with hydrochloric acid containing isatin. The indirubin can be taken up by chloroform and determined by titration with potassium permanganate and sulphuric acid after purification of the chloroform residue. OERUM [4] has also worked out a colorimetric method of estimation based upon BOUMA'S method.

Indol seems also to pass into the urine as a glucuronic acid, *indoxyl-*

[1] Jaffé, Pflüger's Arch., 3; Obermayer, Wien. klin. Wochenschr., 1890; Salkowski, Zeitschr. f. physiol. Chem., 57; Porcher and Hervieux, Zeitschr. f. physiol. Chem., 39.

[2] Rosin, Virchow's Arch., 123; Bouma, Zeitschr. f. physiol. Chem., 27, 30, 32, 39; Wang, *ibid.*, 25, 27, 28; Ellinger, *ibid.*, 38 and 41; Maillard, Bull. soc. chim., Paris (3), 29, and Compt. rend., 136; also L'indoxyle urinaire et les couleurs qui en derivent, Paris, 1903, and Zeitschr. f. physiol. Chem., 41; Hervieux, see Bioch. Centralb., 8, 54.

[3] Krauss, Zeitschr. f. physiol. Chem., 18; Adrian, *ibid.*, 19; Wang, *ibid.*, 25; Salkowski, *ibid.*, 42.

[4] Bouma, Zeitschr. f. physiol. Chem., 32; Oerum, *ibid.*, 45.

glucuronic acid (SCHMIEDEBERG). Such an acid has been found in the urine of animals after the administration of the sodium-salt of *o*-nitrophenylpropiolic acid (G. HOPPE-SEYLER). PORCHER and HERVIEUX [1] have obtained indoxyl sulphuric acid in dogs and asses under similar conditions.

Free indigo, and in fact indirubin as well as indigotin, occur in rare cases in the undecomposed urine. GRÖBER and WANG [2] have recently observed such cases.

Skatoxyl-sulphuric Acid, $C_9H_9NSO_4$, $C_6H_4\langle{}^{C.CH_3}_{NH}\rangle C.O.SO_2.OH$, has not been positively prepared as a constituent of normal urine, but OTTO has once prepared its alkali salt from diabetic urine. Perhaps skatoxyl occurs in normal urine as a conjugated glucuronate (MAYER and NEUBERG [3]), and it is believed that the urine contains a skatol-chromogen from which red and reddish-violet coloring-matters are obtained by decomposition with strong acids and an oxidizing agent.

Skatoxyl-sulphuric acid originates, if it exists in the urine, from skatol, which is formed by putrefaction in the intestine, and which is then conjugated with sulphuric acid after oxidation into skatoxyl. That skatol introduced into the body passes partly as an ethereal-sulphuric acid into the urine has been shown by BRIEGER. Indol and skatol act differently, at least in dogs, indol producing a considerable amount of ethereal-sulphuric acid, while skatol gives only a small quantity (MESTER [4]). Reports on this subject are at variance.

The conditions for the formation of indol and skatol by the putrefaction of proteins in the intestine are decidedly different, according to HERTER, as skatol is produced by other putrefaction bacteria than indol. For example, bacillus coli communis produces indol, but only traces of skatol, while skatol is formed by certain anaerobic putrefactive bacteria. An important intermediary step in the formation of skatol is the indol acetic acid (skatol carboxylic acid, according to SALKOWSKI) and this can also pass into the urine and is the chromogen of the urorosein, according to HERTER.[5]

The potassium salt of skatoxyl-sulphuric acid is crystalline; it dissolves in water, but with difficulty in alcohol. A watery solution becomes deep violet with ferric chloride. The solution becomes red with concentrated hydrochloric acid with the separation of a red precipitate. This precipitate (skatol red) is, after washing with water, insoluble in

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[1] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **14**; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **7** and **8**; Porcher and Hervieux, Journ. de Physiol., **7**.

[2] Gröber, Münch. med. Wochenschr., 1904; Wang, Salkowski's Festschrift, 1904.

[3] Otto, Pflüger's Arch., **33**; Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

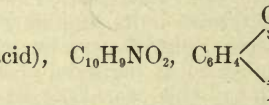
[4] Brieger, Ber. d. deutsch. chem. Gesellsch., **12**, and Zeitschr. f. physiol. Chem., **4**, 414; Mester, *ibid.*, **12**.

[5] Journ. of biol. Chem., **4**.

ether but soluble in amyl alcohol. On distillation with zinc-dust the red pigment gives a strong odor of skatol.

Urines containing skatoxyl are colored dark red to violet by JAFFÉ'S indican test even on the addition of hydrochloric acid alone; with nitric acid they are colored cherry red, and red on warming with ferric chloride and hydrochloric acid. A red coloration of the urine can also be brought about by the appearance of indigo red (indirubin) and a confusion of this pigment can also take place. ROSIN [1] is of the opinion that no skatol chromogen exists in human urine, and that the observations made heretofore were due to a confusion of skatol red with indigo red or urorosein. It cannot be disputed that derivatives of skatol sometimes occur in human urine, and to prevent confusion with indigo red it must be borne in mind that indigo red is soluble in chloroform as well as in ether, while skatol red is insoluble in these solvents. On the contrary skatol red is soluble in amyl alcohol, and this solution shows absorption bands close to the line *D* between it and *E*, corresponding to $\lambda = 577–550$ (PORCHER and HERVIEUX [2]).

In regard to a confusion of skatol red for urorosein it must also be remarked that urorosein may also be a skatol red. The chromogen of urorosein, as HERTER has shown in a case, is identical with indol acetic acid, which passes into skatol on splitting off carbon dioxide. According to HERTER [3] urorosein is not identical with skatol red, although the investigations of STAAL, GROSSER, PORCHER and HERVIEUX [4] indicate that they are identical, and the last two investigators consider them identical, because they both have the same spectrum and the same chemical behavior.

Indol Acetic Acid (skatol carboxylic acid), $C_{10}H_9NO_2$,  .

This acid, whose occurrence in the urine was first shown by SALKOWSKI, is found in the urine in special putrefactive processes in the intestine (HERTER) and in various diseases, especially in cachectic conditions. This is of course dependent upon the fact whether indol acetic acid is the actual chromogen of urorosein, and also whether the experience obtained as to the occurrence of urorosein can be applied to the indol acetic acid. According to WECHSELMANN [5] it occurs (more correctly as urorosein) as traces in normal urine, abundantly in horse urine, and in especially large quantities in cow urine. When introduced into the animal body it appears unchanged in the urine.

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[1] Rosin, Virchow's Arch., **123**.

[2] Zeitschr. f. physiol. Chem., **45**.

[3] Journ. of biol. Chem., **4**.

[4] Staal, Zeitschr. f. physiol. Chem., **46**; Grosser, *ibid.*, **44**; Porcher and Hervieux, *ibid.*, **45**; Compt. rend., **138**, and Journ. de Physiol., **7**.

[5] Salkowski, Zeitschr. f. physiol. Chem., **9**; Wechselmann, cited in Bioch. Centralbl., **5**, 784.

This acid crystallizes in leaves which melt at 165°, and on strongly heating it yields skatol with the splitting off of carbon dioxide. The solution, acidified with hydrochloric acid, when treated with a little ferric chloride solution, becomes cherry red on boiling. With some acid and a little nitrite as well as with hydrochloric acid and chloride of lime the solution becomes red, then cloudy, and a red pigment precipitates. This pigment is soluble in amyl alcohol and gives the above-mentioned absorption bands between *D* and *E*. This red pigment is urorosein.

Urorosein is the name given by NENCKI [1] to a red pigment which occurs in the urine under the conditions mentioned under indol acetic acid. This pigment is not preformed in the urine, but is produced from its chromogen (indol acetic acid) when the urine is treated with hydrochloric acid alone. The urine becomes red. According to HERTER this does not occur in perfectly fresh urine, but only after a formation of nitrite by bacterial action which acts in the reaction as an oxidizing agent. Urorosein differs from indirubin essentially by the same properties as skatol, with which, according to some, it is identical (see above).

Aromatic Oxyacids. In the putrefaction of proteins in the intestine, *paraoxyphenyl-acetic acid* and *paraoxyphenyl-propionic acid* are formed from tyrosine as an intermediate step, and these in great part pass unchanged into the urine. The quantity of these acids is usually very small. They are increased under the same conditions as the phenols, especially in acute phosphorus poisoning, in which the increase is considerable. A small portion of these oxyacids is also combined with sulphuric acid.

Besides these two oxyacids which regularly occur in human urine we sometimes have other oxyacids in urines. To these belong *homogentisic acid* in alcaptonuria, *oxymandelic acid*, found by SCHULTZEN and RIESS in urine in acute atrophy of the liver, *oxyhydroparacoumaric acid*, found by BLENDERMANN in the urine on feeding rabbits with tyrosine, *gallic acid*, which, according to BAUMANN,[2] sometimes appears in horse's urine, and *kynurenic acid* (oxyquinolincarboxylic acid), which up to the present time has been found only in dog's urine. Although all these acids do not belong to the physiological constituents of the urine, still they will be treated in connection with these.

Paraoxyphenylacetic Acid, $C_8H_8O_3$, $C_6H_4\begin{cases}OH\\CH_2.COOH\end{cases}$, and **p-Oxyphenylpropionic Acid** (Hydroparacoumaric Acid), $C_9H_{10}O_3$, $C_6H_4\begin{cases}OH\\CH_2.CH_2COOH\end{cases}$, are crystalline and are both soluble in water and in ether. The one melts at 148° C. and the other at 125° C. Both give a beautiful red coloration on being warmed with MILLON'S reagent.

To detect the presence of these oxyacids proceed in the following way (BAUMANN): Warm the urine for a while on the water-bath with hydrochloric acid in order to drive off the volatile phenols. After cooling shake three times with

[1] Nencki and Sieber, Journ. f. prakt. Chem., (N. F.), **26**.

[2] Schultzen and Riess, Chem. Centralbl., 1869; Blendermann, Zeitschr. f. physiol. Chem., **6**, 267; Baumann, *ibid.*, **6**, 193.

ether, and then shake the ethereal extracts with dilute soda solution, which dissolves the oxyacids, while the residue of the phenols which are soluble in ether remains. The alkaline solution of the oxyacids is now faintly acidified with sulphuric acid, shaken again with ether, the ether removed and allowed to evaporate the residue dissolved in a little water, and the solution tested with MILLON'S reagent. The two oxyacids are best differentiated by their different melting-points. The reader is referred to other works for the method of isolating and separating these two oxyacids.

Homogentisic Acid (Dioxyphenylacetic Acid), $C_8H_4O_4 = C_6H_3\begin{cases}OH(1)\\OH(4)\\CH_2COOH(5)\end{cases}$. This acid, which was discovered by MARSHALL[1] and called by him *glycosuric acid*, was isolated in larger quantities by WOLKOW and BAUMANN in a case of alcaptonuria and carefully studied by them. They called it homogentisic acid because it is a homologue of gentisic acid, and they showed that the peculiar properties of so-called alcaptonuric urine in this case were due to this acid. This acid has later been found in many cases of alcaptonuria by EMBDEN, GARNIER and VOIRIN, OGDEN, GARROD, and many others. *Glycosuric acid*, isolated from alcaptonuric urine by GEYGER,[2] seems to be identical with homogentisic acid.

The quantity of acid eliminated which varies in most cases between 3 and 7 grams per twenty-four hours, and which is higher—14-16 grams—in exceptional cases, is increased by food rich in protein. On the ingestion of tyrosine by persons with alcaptonuria, WOLKOW and BAUMANN and EMBDEN observed a greater quantity of homogentisic acid in the urine. Since LANGSTEIN and E. MEYER showed in a case of alcaptonuria that the quantity of tyrosine in the protein, even when calculated to a maximum, was not sufficient to account for the quantity of homogentisic acid, and that therefore we must admit of another source (the phenylalanine) for the alcapton, FALTA and LANGSTEIN[3] have given a direct proof that homogentisic acid can also be formed from phenylalanine. ABDERHALDEN, BLOCH and RONA[4] have shown that in alcaptonurics the excretion of homogentisic acid is increased by the introduction of tyrosine or phenylalanine in the form of polypeptides, dipeptides as well as tripeptides. The *p*-tyrosine and phenylalanine are quantitatively converted into homogentisic acid, in alcaptonuria (FALTA). The *m*-

[1] The Medical News, Philadelphia, January 8, 1887.

[2] Wolkow and Baumann, Zeitschr. f. physiol. Chem., **15**; Embden, *ibid.*, **17** and **18**; Garnier and Voirin, Arch. de Physiol. (5), **4**; Ogden, Zeitschr. f. physiol. Chem., **20**; Geyger, cited from Embden, l. c., **18**. The literature can be found in Fromherz, Ueber Alkaptonurie, Inaug.-Dist. Freiburg, 1908.

[3] Langstein and Meyer, Deutsch. Arch. f. klin. Med., **78**; Falta and Langstein, Zeitschr. f. physiol. Chem., **37**; Falta, Der Eiweiss-Stoffwechsel bei der Alkaptonurie, Habilitationsschrift, Naumburg a. S., 1904.

[4] Zeitschr. f. physiol. Chem., **52**.

and *o*-tyrosine, on the contrary, are not converted, according to BLUM,[1] into homogentisic acid in alcaptonurics and the dibromtyrosine yields just as little homogentisic acid as the bromine or iodine derivatives of protein bodies (FALTA). According to the investigations of LANGSTEIN and MEYER, and especially of FALTA, different proteins yield varying quantities of homogentisic acid in alcaptonuria, and accordingly larger amounts in proportion as the protein is rich in tyrosine and phenylalanine.

On this account the quotient H(=homogentisic acid):N (nitrogen) is variable on the introduction of different proteins. For example with casein H:N is on an average much higher than with white of egg. In most of the cases of alcaptonuria examined the H:N was equal to 40–50: 100, and with the same alcaptonuric, when no essential change in the diet occurs, the quotient is relatively constant.

WOLKOW and BAUMANN explain the formation of homogentisic acid from tyrosine by an abnormal fermentation in the upper parts of the intestine, but this view has now been generally rejected. The observations of ABDERHALDEN, BLOCH and RONA[2] that glycyl-*l*-tyrosine on subcutaneous injection causes an increased formation of homogentisic acid, disproves this theory, and indicates a formation of homogentisic acid in the tissues. This acid is also burnt in the healthy organism if not too large quantities of the acid are introduced at one time, and it is the general view that alcaptonuria is an anomaly in the protein metabolism.

In order to understand this anomaly and the origin of the homogentisic acid we must call attention to the fact that the investigations of O. NEUBAUER and FALTA, LANGSTEIN and others[3] show that only such aromatic acids are converted, in the body, into homogentisic acid, which have a three-membered side-chain which is substituted by NH_2, OH or H in the α-position to the carboxyl group and not in the β-position, *p*-tyrosine, phenylalanine, phenyl-α-lactic acid and phenyl-pyroracemic acid are such acids. It can be admitted with FALTA that the phenylalanine in the body by deamidation is converted into phenyl-α-lactic acid, $C_6H_5.CH_2.CHOH.COOH$, from which by taking up two hydroxyl groups, dioxyphenyl-α-lactic acid (uroleucic acid), $(OH)_2C_6H_3.CH_2.CHOH.COOH$, is formed, and then from this by oxidation dioxyphenylacetic acid (homogentisic acid), $(OH)_2C_6H_3.CH_2.COOH$, is produced. Tyrosine is also supposed to undergo an analogous transformation whereby a removal of OH groups in the para position must be admitted.

According to NEUBAUER,[4] on the contrary, the tyrosine, as well as

[1] Arch. f. exp. Path. u. Pharm., **59**.

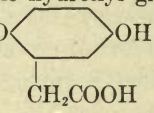
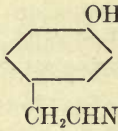
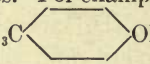
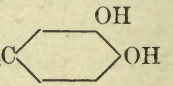
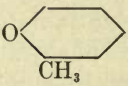
[2] Zeitschr. f. physiol. Chem., **52**.

[3] *Ibid.*, **42**; see also footnote 3, page 698, and Fromherz, l. c.

[4] Cited from Centralbl. f. Physiol., **23**, 76.

the other amino-acids, is first transformed into the corresponding keto-acid, *p*-oxyphenyl pyroracemic acid, $OH.C_6H_4.CH_2.CO.COOH$, which is then oxidized into the corresponding chinol and transformed into hydroquinone pyroracemic acid, $(OH)_2C_6H_3.CH_2.CO.COOH$. The homogentisic acid is derived from this latter by the splitting off of carbon dioxide by oxidative means. Phenylalanine is either changed into phenyl pyroracemic acid or into *p*-oxyphenyl pyroracemic acid with tyrosine as intermediary body and then changed as above stated.

According to the accepted hypothesis the demolition of tyrosine and phenylalanine takes place into homogentisic acid, and the anomaly in the metabolism of alcaptonurics consists in that in these the demolition stops at this point and that the ability to rupture the benzene ring is absent, in the organism, in alcaptonuria.

The difficulties in accepting the assumption of a transformation of tyrosine into homogentisic acid due to the different positions of the hydroxyl groups in the side chain of the two bodies, as shown by the formulæ  (homogentisic acid) and  (tyrosine) do not exist now, since we have learnt of other analogous processes. For example, the oxidation, by Kumagai and Wolffenstein,[1] of paracresol  with potassium persulphate in acid solution. In this manner the expected 3.4 dioxytoluene  was not obtained, but instead homohydroquinone  , and hence a transference of the alkyl group must have occurred.

Garrod,[2] who has observed several cases of alcaptonuria, has also tabulated a large number of cases of alcaptonuria which he finds in the literature, and he shows that the anomaly of the protein metabolism occurs oftener in males than in females, and also that blood relationship of the parents (first cousins) predisposes to alcaptonuria.

On fusing homogentisic acid with alkali it yields gentisic acid (hydroquinone-carboxylic acid) and hydroquinone. When introduced into the intestine of the dog a part is converted into toluhydroquinone, which is eliminated in the form of an ethereal sulphuric acid. Homogentisic

[1] Ber. d. d. chem. Gesellsch., **41**.

[2] Med. chirurg. Transact., 1899 (where all cases up to that time are tabulated); also The Lancet, 1901 and 1902; Garrod and Hele, Journ. of Physiol., **33**.

acid has also been prepared synthetically by BAUMANN and FRÄNKEL, starting with gentisic aldehyde and by NEUBAUER and FLATOW [1] from *o*-oxyphenylglyoxylic acid with hydroquinone glyoxylic acid and hydroquinone glycollic acid as intermediary bodies.

Homogentisic acid crystallizes with 1 mol. of water in large, transparent prismatic crystals, which become non-transparent at the temperature of the room with the loss of water of crystallization. They melt at 146.5–147° C., and are soluble in water, alcohol, and ether, but nearly insoluble in chloroform and benzene. Homogentisic acid is optically inactive and non-fermentable. Its watery solution has the properties of so-called alcaptonuric urine. It becomes greenish brown from the surface downward on the addition of very little caustic soda or ammonia with access of oxygen, and on shaking it quickly becomes dark brown or black. It reduces alkaline copper solutions with even slight heat, and ammoniacal silver solutions immediately in the cold. It does not reduce alkaline bismuth solutions. It gives a lemon-colored precipitate with MILLON'S reagent, which becomes light brick-red on warming. Ferric chloride gives to the solution a blue color which soon disappears. On boiling with concentrated ferric-chloride solution an odor of quinone develops. With benzoyl chloride and caustic soda in the presence of ammonia we obtain the amide of dibenzoylhomogentisic acid, which melts at 204° C., and which can be used in the isolation of the acid from the urine, and also for its detection (ORTON and GARROD). Among the salts of this acid must be mentioned the lead salt containing water of crystallization and 34.79 per cent Pb. This salt melts at 214–215° C.

In order to prepare the acid, heat the urine to boiling, add 5 grams of lead acetate for every 100 cc., filter as soon as the lead acetate has dissolved, and allow the filtrate to stand in a cool place for twenty-four hours until it crystallizes (GARROD). The dried, powdered lead salt is suspended in ether and decomposed by H_2S. After the spontaneous evaporation of the ether the acid is obtained in almost colorless crystals (ORTON and GARROD [2]).

In regard to the quantitative estimation we proceed according to the suggestion of BAUMANN by titrating the acid with a N/10 silver solution. For details of this method the reader is referred to the works of BAUMANN, C. TH. MÖRNER and MITTELBACH, GARROD and HURTLEY. DENIGÈS [3] has suggested another method.

Uroleucic acid, $C_9H_{10}O_5$, is, according to HUPPERT, probably a dioxyphenyllactic acid, $C_6H_3(OH)_2.CH_2.CH(OH).COOH$. This acid was first prepared by KIRK from the urine of children with alcaptonuria, which also contained homogentisic acid. LANGSTEIN and MEYER [4] have also found a small amount of this acid in

[1] Baumann and Fränkel, Zeitschr. f. physiol. Chem., **20**; Neubauer and Flatow, *ibid.*, **52**.

[2] Orton and Garrod, Journ. of Physiol., **27**; Garrod, *ibid.*, **23**.

[3] Mittelbach, Deutsch. Arch. f. klin. Med., **71** (which contains the work of Baumann and Mörner); Garrod and Hurtley, Journ. of Physiol., **33**; Denigès Chem. Cenralbl. 1897, **1**, 338.

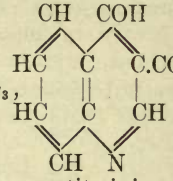
[4] Huppert, Zeitschr. f. physiol. Chem., **23**; Kirk, Brit. Med. Journ., 1886 and 1888; Langstein and Meyer, l. c.

a case of alcaptonuria studied by them. It melts at 130–133° C. Otherwise, in regard to its behavior with alkalies, with access of air, and also with alkaline copper solutions and ammoniacal silver solutions, and also MILLON'S reagent, it is similar to homogentisic acid.

NEUBAUER and FLATOW, who have prepared dioxyphenyl-α-lactic acid synthetically, find that this acid has entirely different properties from the so-called uroleucic acid. GARROD and HURTLEY [1] have also shown that an impure homogentisic acid with a low melting-point is easily obtained, and they suggest the possibility that the earlier reports in regard to uroleucic acid are due to an error.

Oxymandelic acid, $C_8H_8O_4$, paraoxyphenylglycolic acid, $HO.C_6H_4.CH(OH)COOH$ is, as above stated, found in the urine in acute atrophy of the liver. The acid crystallizes in silky needles. It melts at 162° C., dissolves readily in hot water, less in cold water, and readily in alcohol and ether, but not in hot benzene. It is precipitated by basic lead acetate, but not by lead acetate.

Kynurenic acid (γ-oxy-β-quinolincarboxylic acid), $C_{10}H_7NO_3$,



has only been found thus far in dogs' urine, but not always; its quantity is increased by meat feeding. It does not occur in the urine of cats. According to the observations of GLAESSNER and LANGSTEIN the mother-substance seems to be contained among the products of pancreatic digestion which are soluble in alcohol and precipitable by acetone. ELLINGER [2] has been able to show positively that tryptophane is the mother-substance of this acid. By the introduction of tryptophane in the organism he has shown the formation of a kynurenic acid not only in dogs but also in rabbits.

The acid is crystalline, does not dissolve in cold water, rather well in hot alcohol, and yields a barium salt which crystallizes in triangular, colorless plates. On heating it melts and decomposes into CO_2 and kynurin. On evaporation to dryness on the water-bath with hydrochloric acid and potassium chlorate a reddish residue is obtained which on adding ammonia becomes first brownish green and then emerald green (JAFFÉ'S reaction [3]).

Urinary Pigments and Chromogens. The yellow color of normal urine depends perhaps upon several pigments, but in greatest part upon *urochrome*. Besides this the urine seems to contain a very small quantity of *hæmatoporphyrin* as a regular constituent. *Uroerythrin* is also of frequent occurrence in normal urine, but not always. Finally, the excreted urine when exposed to the action of light regularly contains a yellow pigment, *urobilin*, which is derived from a chromogen, *urobilinogen*,

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[1] Journ. of Physiol., **36**.

[2] Glaessner and Langstein, Hofmeister's Beiträge, **1**; Ellinger, Ber. d. d. chem. Gesellsch., **37**, 1804, and Zeitschr. f. physiol. Chem., **43**. The older literature on kynurenic acid may be found in Josephsohn, Beiträge zur Kenntnis der Kynurensäure ausscheidung beim Hunde, Inaug.-Dissert., Königsberg, 1898.

[3] Zeitschr. f. physiol. Chem., **7**. In regard to kynurenic acid, see also Huppert-Neubauer, 10. Aufl., and Mendel and Jackson, Amer. Journ. of Physiol., **2**; Mendel and Schneider, *ibid.*, **5**; Camps, Zeitschr. f. physiol. Chem., **33**.

by the action of light (SAILLET) and air (JAFFÉ, DISQUÉ [1] and others). Besides this chromogen, urine contains various other bodies from which coloring matters may be produced by the action of chemical agents. Humin substances (perhaps in part from the carbohydrates of the urine) may be formed by the action of acids (v. UDRÁNSZKY) without regard to the fact that such substances may sometimes originate from the reagents used, as from impure amyl alcohol (v. UDRÁNSZKY[2]). To these humin bodies developed by the action of acid in normal urine when exposed to the air must be added the *urophain* of HELLER, the various *uromelanins* and other bodies described by different investigators (PLÓSZ, THUDICHUM, SCHUNCK [3]). Indigo blue (*uroglaucin* of HELLER, *urocyanin*, *cyanurin*, and other coloring matters of earlier investigators [4]) is split off from the indoxyl-sulphuric acid or indoxyl-glucuronic acid. Red coloring matter may be formed from the conjugated indoxyl and skatoxyl acids, and *urohodin* (HELLER), *urorubin* (PLÓSZ), *urohæmatin* (HARLEY), and perhaps also *urorosein* (NENCKI and SIEBER [5]) probably have such an origin.

We cannot discuss more in detail the different coloring matters obtained as decomposition products from normal urine. Hæmatoporphyrin has already been referred to in a previous chapter (VI) and will best be described in connection with the pathological pigments. It only remains to describe urochrome, urobilin, and uroerythrin.

Urochrome is the name given by GARROD to the yellow pigment of the urine. THUDICHUM [6] had previously given the same name to a less pure pigment isolated by himself. The accounts as to the composition and properties of urochrome differ so considerably that it is questionable whether anybody has ever had this pigment in a pure form. Urochrome is free from iron, but contains nitrogen. DOMBROWSKY found 11.15 per cent nitrogen, HOHLWEG found 9.89 per cent nitrogen, and KLEMPERER found only 4.2 per cent nitrogen. According to DOMBROWSKY urochrome contains about 5 per cent sulphur, while other investigators like HOHLWEG, SALOMONSEN, and MANCINI found that is was free from sulphur.[7] According to GARROD it stands in

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[1] Jaffé, Centralbl. f. d. med. Wissensch., 1868 and 1869, and Virchow's Arch., **47**; Disqué, Zeitschr. f. physiol. Chem., **2**; Saillet, Revue de médecine, **17**, 1897.

[2] v. Udránszky, Zeitschr. f. physiol. Chem., **11**, **12**, and **13**.

[3] Plósz, Zeitschr. f. physiol. Chem., **8**; Thudichum, Brit. Med. Journ., **201**, and Journ. f. prakt. Chem., **104**; Schunck, cited from Huppert-Neubauer, 10. Aufl., 509.

[4] See Huppert-Neubauer, 161.

[5] In regard to this and other red pigments, see Huppert-Neubauer, 593 and 597; Nencki and Sieber, Journ. f. prakt. Chem. (2), **26**.

[6] Garrod, Proc. Roy. Soc., **55**; Thudichum, l. c.

[7] Dombrowsky, Zeitschr. f. physiol. Chem., **54**; Hohlweg, Bioch. Zeitschr., **13**; Salomonsen, *ibid.*, **13**; Mancini, *ibid.*, **13**; Klemperer, Berl. klin. Wochenschr., **40**.

close relation to urobilin and is transformed into urobilin by the action of "active" acetaldehyde, while RIVA [1] claims to have obtained a body similar to urochrome by the oxidation of urobilin by permanganate. This relation of the two pigments is denied by DOMBROWSKY. Nevertheless it is known that urochrome gives the pyrrol reactions under certain conditions. SALOMONSEN and especially MANCINI have prepared a bromine derivative of urochrome whose mother-substance is called uropyrryl by MANCINI, and has the approximate formula $C_{34}H_{47}N_7O_{12}$.

Urochrome, as obtained thus far, is amorphous, brown, readily soluble in water and ordinary alcohol, but less soluble in absolute alcohol. It dissolves but slightly in acetic ether, amyl alcohol, and acetone, while it is insoluble in ether, chloroform, and benzene. Urochrome is precipitated by lead acetate, silver nitrate, mercuric acetate, phosphotungstic and phosphomolybdic acids. On saturating the urine with ammonium sulphate a great part of the urochrome remains in solution. It does not show any absorption-bands and does not fluoresce after the addition of ammonia and zinc chloride. Urochrome is very readily decomposed, by the action of acids, with the formation of brown substances.

Urochrome can be prepared according to a rather complicated method which is based upon the fact that the substance remains in great part in solution on saturating the urine with ammonium sulphate. If the proper quantity of alcohol is added to the filtrate, a clear, yellow alcoholic layer forms on the salt solution, which contains the urochrome and which can be used for the further preparation of the latter (see GARROD, O. BOCCHI[2]). KLEMPERER, on the contrary, removes the pigment from the urine by means of animal charcoal, washes it with water to remove the indican and other bodies, and then extracts with alcohol and uses this alcoholic extract for the further purification according to GARROD. HOHLWEG, SALOMONSEN and MANCINI also remove the pigment from the urine, which has previously been precipitated by calcium or barium salts, by means of animal charcoal. DOMBROWSKY uses an entirely different method which is based upon the precipitation of the urochrome by copper acetate. In regard to the details of these different methods we refer to the original works.

DOMBROWSKY, BROWINSKI and DOMBROWSKY [3] have worked out a quantitative method for estimating urochrome, but its value is dependent upon a further investigation as to the purity and composition of the urochrome used by them. On this account the results found by these investigators will not be given. The urochrome can be quantitatively estimated, according to KLEMPERER, by a colorimetric method, using a solution of true yellow G. If 0.1 gram of this dye is dissolved in 1 liter of water and 5 cc. of this solution diluted to 50 cc. with water, then

[1] Garrod, Journ. of Physiol., **21** and **29**; Riva, cited from Huppert-Neubauer, 524.

[2] Garrod, l. c.; Bocchi, Hofmeister's Beiträge, **11**.

[3] Dombrowsky, Zeitschr. f. physiol. Chem., **54**, with Browinski, Bull. Acad. d. d. scien. Cracovie, 1908; Klemperer, l. c.

this solution has the same color and shade as a 0.1 per cent urochrome solution. The urine must be diluted with water until it has the same depth of color. The comparison is performed in vessels with parallel walls.

Urobilin is the pigment first isolated from the urine by JAFFÉ,[1] and which is characterized by its strong fluorescence and by its absorption-spectrum. Various investigators have prepared, from the urine, by different methods, pigments which differed slightly from each other but behaved essentially like JAFFÉ'S urobilin. Thus different urobilins have been suggested, such as normal, febrile, physiological, and pathological urobilins.[2] The possibility of the occurrence of different urobilins in the urine cannot be denied; but as urobilin is a readily changeable body and difficult to purify from other urinary pigments, the question as to the occurrence of different urobilins must still be considered open. According to SAILLET[3] no urobilin exists originally in human urine, but only the mother-substance of the same, urobilinogen, from which the urobilin is formed in the excreted urine by the influence of light.

Urobilin-like bodies, so-called *urobilinoids*, have been prepared from bile-pigments as well as blood-pigments, and indeed by oxidation as well as by reduction. MALY obtained his hydrobilirubin by the reduction of bilirubin with sodium amalgam, and DISQUÉ obtained a product which is still more similar to urobilin, while STOKVIS prepared, by the oxidation of cholecyanin, with a little lead peroxide, a choletelin, which acted very much like urobilin. HOPPE-SEYLER, LE NOBEL, NENCKI and SIEBER have obtained urobilinoid bodies by the reduction of hæmatin and hæmatoporphyrin with tin or zinc and hydrochloric acid, while MACMUNN[4] obtained, by the oxidation of hæmatin with hydrogen peroxide in alcohol containing sulphuric acid a pigment, which seemed to be identical with urinary urobilin. It is apparent that all these urobilins cannot be identical.

Many investigators declare that urobilin is identical with hydrobilirubin, but according to the researches of HOPKINS and GARROD[5] this view is not correct, because, irrespective of other small differences, each body has an essentially distinct composition. Hydrobilirubin contains C 64.68, H 6.93,

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[1] Centralbl. f. d. med. Wissensch., 1868 and 1869, and Virchow's Arch., **47**.

[2] See MacMunn, Proc. Roy. Soc., **31** and **35**; Ber. d. deutsch. chem. Gesellsch., **14**, and Journ. of Physiol., **6** and **10**; Bogomoloff, Maly's Jahresber., **22**; Eichholz, Journ. of Physiol., **14**; Ad. Jolles, Pflüger's Arch., **61**.

[3] Revue de médecine, **17**, 1897.

[4] Maly, Ann. d. Chem. u. Pharm., **163**; Disqué, Zeitschr. f. physiol. Chem., **2**; Stokvis, Centralbl. f. d. med. Wissensch., 1873, 211 and 449; Hoppe-Seyler, Ber. d. deutsch. chem. Gesellsch., **7**; Le Nobel, Pflüger's Arch., **40**; Nencki and Sieber, Monatshefte f. Chem., **9**, and Arch. f. exp. Path. u. Pharm., **24**; MacMunn, Proc. Roy. Soc., **31**.

[5] Journ. of Physiol., **22**.

N 9.22 (Maly), while urinary urobilin, on the contrary, contains C 63.46, H 7.67, N 4.09 per cent. The urobilin from feces, *stercobilin*, has the same composition as urinary urobilin with 4.17 per cent nitrogen.

Urinary urobilin may not be identical with hydrobilirubin, but this does not exclude the possibility that urobilin, according to the generally admitted view, is derived from bilirubin (although not by simple reduction and taking up of water) in the intestine. Several physiological as well as clinical observations [1] point to the truth of this theory, among which we must mention the regular appearance in the intestinal tract of stercobilin, undoubtedly derived from the bile-pigments and having the same composition as urinary urobilin, the absence of urobilin in the urine of new-born infants as well as on the complete exclusion of bile from the intestine, and also the increased elimination of urobllin with strong intestinal putrefaction. On the other hand there are investigators who, basing their opinion on clinical observations, deny the intestinal origin of urobilin and claim that the urobilin is derived from a transformation of the bilirubin elsewhere than in the intestine, by an oxidation of the bile-pigment or by a transformation of the blood-pigments.[2] The possibility of a different mode of formation of urinary urobilin in disease is not to be denied; but there is no doubt that this pigment is formed from the bile-pigments in the intestine under physiological conditions.

Urobilin does not occur in the urine of all animals, and according to Fromholdt it is absent in the urine as well as in the feces of rabbits. Biffi [3] finds abundance of urobilin or urobilinogen in the blood of human cadavers, while it is normally absent in the blood during life. In cases with inflammation of the lungs it occurs according to Biffi in the blood during life.

The quantity of urobilin in the urine under physiological conditions varies widely. Saillet found 30–130 milligrams and G. Hoppe-Seyler 80–140 milligrams in one day's urine.

There are numerous observations on the elimination of urobilin in disease, especially by Jaffé, Disqué, Gerhardt, G. Hoppe-Seyler,[4]

[1] See Fr. Müller, Schles. Gesellsch. f. vaterl. Kultur, 1892; D. Gerhardt, "Ueber Hydrobilirubin und seine Bezieh. zum Ikterus" (Inaug.-Diss., Berlin, 1889); Beck, Wien. klin. Wochenschr., 1895; Harley, Brit. Med. Journ., 1896; Fischler, Zeitschr. f physiol. Chem., 48.

[2] In regard to the various theories as to the formation of urobilin, see Harley, Brit. Med. Journ., 1896; A. Katz., Wien. med. Wochenschr., 1891, Nos. 28–32; Grimm, Virchow's Arch., **132**; Zoja, Conferenze cliniche italiane, Ser. 1a, **1**; Hildebrandt, Zeitschr. f. klin. Med., **59**; Biffi, Boll. d. scienc. med. di Bologna (8), anno 78, 7.

[3] Fromholdt, Zeitschr. f. physiol. Chem., **59**; Biffi, Folia hæmatol, **4**, and l. c. Boll., **78**.

[4] In regard to the literature on this subject we refer the reader to D. Gerhardt, "Ueber Hydrobilirubin und seine Beziehungen zum Ikterus" (Berlin, 1889), and also G. Hoppe-Seyler, Virchow's Arch., **124**.

and others. The quantity is increased in hemorrhage and in diseases where the blood-corpuscles are destroyed, as is the case after the action of certain blood-poisons, such as antifebrin and antipyrine. It is also increased in fevers, cardiac diseases, lead colic, atrophic cirrhosis of the liver, and is especially abundant in so-called urobilin icterus.

The properties of urobilin may vary, depending upon the method of preparation and the character of the urine used; therefore only the most important properties will be given. Urobilin is amorphous, brown, reddish brown, red, or reddish yellow, depending upon the method of preparation. It dissolves readily in alcohol, amyl alcohol, and chloroform, but less readily in ether or acetic ether. It is less soluble in water, but the solubility is augmented by the presence of neutral salts. It may be completely precipitated from the urine by saturating with ammonium sulphate, especially after the addition of sulphuric acid (MÉHU [1]). It is soluble in alkalies, and is precipitated from the alkaline solution by the addition of acid. It is partly dissolved by chloroform from an acid (watery-alcoholic) solution; alkali solutions remove the urobilin from the chloroform. The neutral or faintly alkaline solutions are precipitated by certain metallic salts (zinc and lead), but not by others, such as mercuric sulphate. Urobilin is precipitated from the urine by phosphotungstic acid. It does not give GMELIN'S test for bile-pigments. It gives, on the contrary, a reaction which may be mistaken for the biuret test, by the action of copper sulphate and alkali.[2]

Neutral alcoholic urobilin solutions are in strong concentration brownish yellow, in great dilution yellow or rose-colored. They have a strong green fluorescence. The acid alcoholic solutions are brown, reddish yellow, or rose-red, according to concentration. They are not fluorescent, but show a faint absorption-band, γ, between b and F, which borders on F, or in greater concentration extends over F. The alkaline solutions are brownish yellow, yellow, or (the ammoniacal) yellowish green, according to concentration. If some zinc-chloride solution is added to an ammoniacal solution of the pigment it becomes red and shows a beautiful green fluorescence. This solution, as also that made alkaline with fixed alkalies, shows a darker and more sharply defined band, δ, between b and F, almost midway between E and F. If a sufficiently concentrated solution of urobilin alkali is carefully acidified with sulphuric acid it becomes cloudy and shows a second band exactly at E and connected with γ by a shadow (GARROD and HOPKINS, SAILLET [3]).

Urobilinogen is colorless or is only slightly colored. Like urobilin it

[1] Journ. de Pharm. et Chim., 1878, cited from Maly's Jahresber., 8.

[2] See Salkowski, Berlin. klin. Wochenschr., 1897, and Stokvis, Zeitschr. f. Biologie, **34**.

[3] Garrod and Hopkins, Journ. of Physiol., **20**; Saillet, l. c.

is precipitated from the urine by saturating with ammonium sulphate. According to SAILLET it may be extracted by acetic ether from urine acidified with acetic acid. It also dissolves in chloroform, ethyl-ether and amyl-alcohol. It shows no absorption-bands and is readily converted into urobilin by the influence of sunlight and oxygen, and, according to NEUBAUER and BAUER,[1] gives the EHRLICH reaction with dimethylamido-benzaldehyde and hydrochloric acid (see below).

In preparing urobilin from normal urine, precipitate the urine with basic lead acetate (JAFFÉ), wash the precipitate with water, dry at the ordinary temperature, then boil it with alcohol, and decompose it when cold with alcohol containing sulphuric acid. The filtered alcoholic solution is diluted with water, saturated with ammonia, and then treated with zinc-chloride solution. This new precipitate is washed free from chlorine with water, boiled with alcohol, dried, dissolved in ammonia, and this solution precipitated with sugar of lead. This precipitate, which is washed with water and boiled with alcohol, is decomposed by alcohol containing sulphuric acid, the filtered alcoholic solution is mixed with ½ vol. chloroform, diluted with water, and shaken repeatedly, but not too energetically. The urobilin is taken up by the chloroform. This last is washed once or twice with a little water and then distilled, leaving the urobilin. The pigment may be precipitated directly from the urine rich in urobilin by ammonia and zinc chloride, and the precipitate treated as above described (JAFFÉ).

The method suggested by MÉHU (precipitation with ammonium sulphate) has been modified by GARROD and HOPKINS in that they first remove the uric acid by saturating with ammonium chloride and then saturating the filtrate with ammonium sulphate. In regard to small details, and to a second method suggested by these experimenters, we refer to the original work.[2]

SAILLET extracts the urobilinogen from the urine by shaking with acetic ether, using a kerosene-oil light. In regard to this and other methods we must refer to other hand-books.

The color of the acid or alkaline solution, the beautiful fluorescence of the ammoniacal solution treated with zinc chloride, and the absorption-bands of the spectrum, all serve as means of detecting urobilin. In fever-urines the urobilin may be detected, directly, or after the addition of ammonia and zinc chloride, by its spectrum. It may also sometimes be detected in normal urine, either directly or after the urine has stood exposed to the air until the chromogen has been converted into urobilin. If it cannot be detected by means of the spectroscope, then the urine may be treated with a mineral acid and shaken with ether or, still better, with amyl alcohol. The amyl-alcohol solution is, either directly or after addition of a strongly ammoniacal alcoholic solution of zinc chloride, tested spectroscopically. According to SCHLESINGER[3] it can be readily detected if the urine is precipitated by an equal volume

[1] Neubauer, cited from Centralbl. f. Physiol., **19**, 145; Bauer, cited from Biochem. Centralbl., **4**, 390.

[2] Journ. of Physiol., **20**.

[3] Deutsch. med. Wochenschr., 1903.

of a 10 per cent solution of zinc acetate in absolute alcohol. Disturbing bodies are here precipitated and the filtrate gives the fluorescence directly, and also the spectrum. GRIMBERT[1] has given another comparatively simple method.

The detection of urobilin in feces can be accomplished in various ways and very simply by the aid of alcoholic extracts as suggested by SALKOWSKI.[2]

In the quantitative estimation of urobilin we proceed as follows, according to G. HOPPE-SEYLER:[3] 100 cc. of the urine are acidified with sulphuric acid and saturated with ammonium sulphate. The precipitate is collected on a filter after some time, washed with a saturated solution of ammonium sulphate, and repeatedly extracted with equal parts of alcohol and chloroform after pressing. The filtered solution is treated with water in a separatory funnel until the chloroform separates well and becomes clear. The chloroform solution is evaporated on the water-bath in a weighed beaker, the residue dried at 100° C., and then extracted with ether. The ethereal extract is filtered, the residue on the filter dissolved in alcohol, and transferred to the beaker and evaporated, then dried and weighed. According to this method G. HOPPE-SEYLER found 0.08–0.14 gram of urobilin in one day's urine of a healthy person, or an average of 0.123 gram.

Urobilin may also be determined spectrophotometrically according to FR. MÜLLER or SAILLET.[4] SAILLET found that the limit for the perceptibility of the absorption-bands of an acid-urobilin solution lies in a concentration of 1 milligram of urobilin in 22 cc. of solution when the thickness of the layer of fluid is 15 mm. In a quantitative estimation the urobilin solution is diluted to this limit and then the quantity of urobilin calculated from the extent of dilution. The freshly voided urine, shielded from light, is acidified with acetic acid, completely extracted in kerosene-oil light with acetic ether, and the dissolved urobilinogen oxidized to urobilin with nitric acid. On the addition of ammonia and shaking with water the urobilin passes into the watery solution. This is acidified with hydrochloric acid and diluted until the above limit is reached.

Uroerythrin is the pigment which often gives the beautiful red color to the urinary sediments (*sedimentum lateritium*). It also frequently occurs, although only in very small quantities, dissolved in normal urines. The quantity is increased after great muscular activity, after profuse perspiration, immoderate eating, or partaking of alcoholic drinks, as well as after digestive disturbances, fevers, circulatory disturbances of the liver, and in many other pathological conditions.

Uroerythrin, which has been especially studied by ZOJA, RIVA, and GARROD,[5] has a pink color, is amorphous, and is very quickly destroyed by light, especially when in solution. The best solvent is amyl alcohol;

[1] See Chem. Centralbl., 1904, **1**, 1623.

[2] Arbeit. aus. d. pathol. Inst. Berlin. Festschr., 1906.

[3] Virchow's Arch., **124**.

[4] Fr Müller, see Huppert-Neubauer, 861; Saillet, l. c.

[5] Zoja, Arch. ital. di clinica med., 1893, and Centralbl. f. d. med. Wissensch., 1892; Riva, Gaz. med di Torino, Anno 43, cited from Maly's Jahresber., **24**; Garrod, Journ. of Physiol., **17** and **21**.

acetic ether is not so good, and alcohol, chloroform, and water are even less valuable. The very dilute solutions show a pink color; but on greater concentration they become reddish orange or bright red. They do not fluoresce either directly or after the addition of an ammoniacal solution of zinc chloride; but they have a strong absorption, beginning in the middle between *D* and *E* and extending to about *F*, and consisting of two bands which are connected by a shadow between *E* and *b*. Concentrated sulphuric acid colors a uroerythrin solution a beautiful carmine red; hydrochloric acid gives a pink color. Alkalies make its solutions grass green, and often a play of colors from pink to purple and blue is observed. PORCHER and HERVIEUX [1] claim that uroerythrin is a skatol pigment.

In preparing uroerythrin according to GARROD, the sediment is dissolved in water at a gentle heat and saturated with ammonium chloride, which precipitates the pigment with the ammonium urate. This is purified by repeated solution in water and precipitation with ammonium chloride until all the urobilin is removed. The precipitate is finally extracted on the filter in the dark with warm water, filtered, then diluted with water, any hæmatoporphyrin remaining being removed by shaking with chloroform; the precipitate is then faintly acidified with acetic acid and shaken with chloroform, which takes up the uroerythrin. The chloroform is evaporated in the dark at a gentle heat.

Volatile fatty acids, such as formic acid, acetic acid, and perhaps also butyric acid, occur under normal conditions in human urine (v. JAKSCH), also in that of dogs and herbivora (SCHOTTEN). The acids poorest in carbon, such as formic acid and acetic acid, are more constant in the body than those richer in carbon, and therefore the relatively greater part of these pass unchanged into the urine (SCHOTTEN). Normal human urine contains besides these bodies others which yield acetic acid when oxidized by potassium dichromate and sulphuric acid (v. JAKSCH). The quantity of volatile fatty acids in normal urine calculated as acetic acid is, according to v. JAKSCH, 0.008–0.009 gram per twenty-four hours; according to v. ROKITANSKY, 0.054 gram; and according to MAGNUS-LEVY 0.060 gram. The quantity is increased by exclusively farinaceous food (ROKITANSKY), in fever and in certain diseases, while in others it is diminished (v. JAKSCH, ROSENFELD). Large amounts of volatile fatty acids are produced in the alkaline fermentation of the urine, and the quantity is 6–15 times as large as in normal urine (SALKOWSKI [2]). *Non-volatile fatty acids* have been detected as normal constituents of urine by K. MÖRNER and HYBBINETTE.[3]

Paralactic Acid. It is claimed that this acid occurs in the urine of healthy persons after very fatiguing marches (COLASANTI and MOSCATELLI). It is found in larger amounts in the urine in acute phosphorus-poisoning or acute yellow atrophy of the liver (SCHULTZEN and RIESS), in pregnancy (UNDERHILL), and especially abundant in eclampsia (ZWEIFEL and others). According to the investigations of HOPPE-SEYLER, ARAKI, and v. TERRAY lactic acid passes into the urine as soon as the supply of oxygen is decreased in any way, and this probably explains the occurrence of lactic acid in the urine after epileptic attacks (INOUYE and SAIKI). MINKOWSKI [4] has shown that lactic acid occurs in the urine in large quantities on the extirpation of the liver of birds.

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[1] Journ. de Physiol., **7**.

[2] v. Jaksch, Zeitschr. f. physiol. Chem., **10**; Schotten, *ibid.*, **7**; Rokitansky, Wien. med. Jahrbuch, 1887; Salkowski, Zeitschr. f. physiol. Chem., **13**; Magnus-Levy, Salkowski's Festschrift, 1904; Rosenfeld, Deutsch. med. Wochenschr., **29**.

[3] Skand. Arch. f. Physiol., **7**.

[4] Colasanti and Moscatelli, Moleschott's Untersuch., **14**; Schultzen and Riess,

Glycerophosphoric acid occurs as traces in the urine,[1] and it is probably a decomposition product of lecithin. The occurrence of *succinic acid* in normal urine is a subject of discussion.

Carbohydrates and Reducing Substances in the Urine. The occurrence of *dextrose*, as traces, in normal urine is highly probable, as the investigations of BRÜCKE, ABELES, and v. UDRÁNSZKY show. The last investigator has also shown the habitual occurrence of carbohydrates in the urine, and their presence has been positively proven by the investigations of BAUMANN and WEDENSKI, and especially by BAISCH. Besides dextrose normal urine contains, according to BAISCH, another not well-studied variety of sugar; according to LEMAIRE, probably isomaltose is present, and besides this a dextrin-like carbodydrate (animal gum), as shown by LANDWEHR, WEDENSKI, and BAISCH. The quantity of carbohydrates eliminated under normal conditions in the twenty-four hours' urine and determined by the benzoylation method, which is perhaps not sufficiently trustworthy, varies considerably between 1.5 and 5.09 grams.[2]

The precipitate obtained from concentrated urine by the aid of alcohol and whose nitrogen (colloidal nitrogen according to SALKOWSKI) in normal urine amounts to 2.34–4.08 per cent of the total nitrogen and in pathological urines to 8–9 per cent, and in a case of acute yellow atrophy of the liver to 21.8 per cent, contains, SALKOWSKI[3] claims, a nitrogenous carbohydrate which has strong reducing action upon alkaline copper solutions after cleavage with hydrochloric acid.

Besides traces of sugar and the reducing substances previously mentioned, uric acid and creatinine, the urine contains still other bodies of this character. These latter are partly conjugated compounds of *glucuronic acid*, $C_6H_{10}O_7$, which is closely allied to dextrose. The reducing power of normal urine corresponds, according to various investigators, to 1.5–5.96 p. m. dextrose. That portion of the reduction belonging to dextrose alone is equal to 0.1–0.6 p. m. LAVESON[4] believes that of the total reduction 17.8 per cent is due to sugar, 26.3 per cent to creatinine, 7.8 per cent to uric acid, and the remainder, nearly 50 per cent, is caused by chiefly unknown bodies.

Chem. Centralbl., 1869; Underhill, Journ. of biol. Chem., **2**; Zweifel, Arch. f. Gynäkol, **76**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**, **19**. See also Irisawa, *ibid.*, **17**; v. Terray, Pflüger's Arch., **65**; Schütz, Zeitschr. f. physiol. Chem., **19**; Inouye and Saiki, *ibid.*, **37**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**.

[1] See Pasqualis, Maly's Jahresber., **24**.

[2] Lemaire, Zeitschr. f. physiol. Chem., **21**; Baisch, *ibid.*, **18**, **19**, and **20**. In these as well as in Treupel, *ibid.*, **16**, the works of other investigators are cited. See also v. Alfthan, Deutsch. med. Wochenschr., **26**.

[3] Berlin. klin. Wochenschr., 1905.

[4] Flückiger, Zeitschr. f. physiol. Chem., **9**; Laveson, Bioch. Zeitschr., **4**; see also Huppert-Neubauer, page 72.

Several new methods for the determination of the reducing power of the urine have been suggested.[1]

Conjugated glucuronates occur, as indicated by FLÜCKIGER and first positively shown by MAYER and NEUBERG,[2] in very small amounts in normal urine. They occur chiefly as phenol- and only very small amounts of indoxyl- or skatoxylglucuronates. The quantity of glucuronic acid obtained from the conjugated glucuronates is estimated as 0.04 p. m. by MAYER and NEUBERG. Besides these conjugated glucuronates perhaps the urine sometimes contains the urea glucuronic acid, the ureidoglucuronic acid prepared synthetically by NEUBERG and NEIMANN.[3]

Very large amounts of these conjugated glucuronates occur in the urine, on the other hand, after partaking of various therapeutic agents and other substances, such as chloral hydrate, camphor, naphthol, borneol, turpentine, morphine, and many other substances. The elimination of glucuronic acid may be markedly increased in severe disturbances of the respiration, severe dyspnœa, in diabetes mellitus, and by the direct introduction of large amounts of dextrose. According to P. MAYER, as stated on page 215, in the oxidation of dextrose a part of it forms glucuronic acid, hence it is to be expected that the glucuronic acid can in part be derived from the dextrose. As a conjugation of the glucuronic acid with other bodies, such as aromatic atomic complexes, prevents the combustion of this acid in the animal body, it ought to follow that after the introduction of such an atomic complex in the body during a glycosuria a corresponding reduction of the glucose elimination would take place with the increased excretion of conjugated glucuronates. In order to prove this possibility O. LOEWI[4] fed dogs with camphor during phlorhizin diabetes and found that the above expectation was not realized. Although large quantities of campho-glucuronic acid were excreted, the sugar excretion was only slightly diminished and not in proportion to the quantity of conjugated glucuronate excreted. These negative results are contradicted by the positive results obtained by PAUL MAYER.[5] Rabbits normally convert almost all the camphor introduced into conjugated glucuronic acid. MAYER claims that if we allow a rabbit to starve several days the animal becomes so poor in the mother-substance (glycogen) yielding the glucuronic acid that the introduction of camphor only brings about an elimination of small quantities of glucuronic acid. By the simultaneous administration of camphor and dextrose while

[1] See Rosin, Münch. med. Wochenschr., 46; Niemilowicz, Zeitschr. f. physiol. Chem., 33; Niemilowicz with Gittelmacher-Wilenko, *ibid.*, 36, and Hélier, Compt. rend., 129.

[2] Flückiger, l. c.; Mayer and Neuberg, Zeitschr. f. physiol. Chem., 29.

[3] Zeitschr. f. physiol. Chem., 44.

[4] Arch. f. exp. Path. u. Pharm., 47.

[5] Zeitschr. f. klin. Med., 47.

starvation is going on, the elimination of glucuronic acid rises again to the same height as it was before the starvation period. This shows that the sugar had conjugated itself with the camphor as glucuronic acid. HILDEBRANDT [1] has also made experiments showing that glucuronic acid can very likely be formed from sugar. The observations of MAYER are not substantiated by the recent investigations of FENYVESSY,[2] and the observers do not agree on this question.

The conjugated glucuronic acids are formed, based upon the investigations of SUNDWIK, FISCHER and PILOTY,[3] by a combination taking place first between the conjugator and the dextrose by means of the aldehyde group, and then the end alcohol group, CH_2OH, is oxidized to COOH. The conjugated glucuronic acids, at least in most cases, seem to be constructed after the glucoside type, a view which has received further support by the synthesis of phenolglucuronic acid and euxanthonglucuronic acids by NEUBERG and NEIMANN.[4] Based upon their cleavage (as far as they have been investigated) by kephir lactase and emulsin, but not by yeast lactase (NEUBERG and WOHLGEMUTH [5]), the conjugated glucuronic acids must belong to the β-series of glucosides. We also know of certain conjugated glucuronates that are constructed upon the ester type, namely, the dimethylaminobenzoicglucuronate, discovered by JAFFÉ and also the benzoicglucuronic acid, after feeding benzoic acid (MAGNUS-LEVY).[6]

According to the body with which they are conjugated the glucuronates vary in behavior. On taking up water they split into glucuronic acid and the conjugated group and this is brought about by boiling with a dilute mineral acid. They are precipitated by basic lead acetate or by basic lead acetate and ammonia. Most of the conjugated glucuronic acids do not have a direct reducing action but are reducing after hydrolysis. Certain of them, and to this group belong especially those acids of the ester type, reduce copper oxide and certain other metallic oxides in alkaline solution directly, and hence cause errors in the investigations of the urine for sugar. The conjugated acids of the glucoside type rotate the plane of polarized light to the left, while the glucuronic acid itself is dextrorotatory. The conjugated acids of the ester type, which as a rule are less stable, rotate the ray of polarized light to the right. As the detection of conjugated glucuronic acids is connected with the tests for sugar in the urine, we will treat of this in connection with these tests.

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[1] Arch. f. exp. Path. u. Pharm., **44**.

[2] See Maly's Jahresber., **34**.

[3] E. Sundwik, Akademische Abhandlung Helsingfors, 1886; see also Maly's Jahresber., **16**, 76; Fischer and Piloty, Ber. d. d. chem. Gesellsch., **24**.

[4] Zeitschr. f. physiol. Chem., **44**.

[5] See Neuberg, Ergebnisse der Physiologie, Bd. **3**, Abt. 1, 444.

[6] Jaffé, Zeitschr. f. physiol. Chem., **43**; Magnus-Lavy, Bioch., Zeitschr., 6.

Organic combinations containing sulphur of unknown kind, which may in small part consist of *sulphocyanides*, 0.04 (Gscheidlen) to 0.11 p. m. (I. Munk [1]), *cystine* or bodies related to it, *taurine derivatives*, *chrondroitin-sulphuric acid* and *protein bodies*, but in greater part are made up of *antoxyproteic acid*, *oxyproteic acid*, *alloxyproteic acid*, and *uroferric acid*, are found in human as well as in animal urines. The sulphur of these mostly unknown combinations has been called "neutral," to differentiate it from the "acid" sulphur of the sulphate and ethereal-sulphuric acid (Salkowski [2]). The neutral sulphur in normal urine as determined by Salkowski is 15 per cent, by Stadthagen 13.3–14.5 per cent, and by Lépine 20 per cent, and Harnack and Kleine [3] 19–24 per cent of the total sulphur. In starvation, according to Fr. Müller, with insufficient supply of oxygen (Reale and Boeri, Harnack and Kleine), as in chloroform narcosis (Kast and Mester), as also after the introduction of sulphur (Presch and Yvon [4]), the quantity of neutral sulphur is increased. The quantity of neutral sulphur varies, according to Benedict, within rather narrow limits and especially, according to Folin, it is dependent to a less degree than the sulphate excretion upon the extent of the protein metabolism. The relation between the neutral and acid sulphur depends in the first place upon the extent of the sulphuric-acid excretion. According to Harnack and Kleine,[5] the relation of the oxidized sulphur to the total sulphur changes always in the same way as the relation of the nitrogen of the urea to the total nitrogen. The more unoxidized sulphur is eliminated the more abundant are the nitrogen compounds, not urea, in the urine—a statement which coincides with recent observations showing that the neutral sulphur originates chiefly from the different proteic acids, and the uroferric acid.

According to Lépine, a part of the neutral sulphur is more readily oxidized (directly with chlorine or bromine) into sulphuric acid than the other, which is only converted into sulphuric acid after fusing with potash and saltpeter. The investigations of W. Smith [6] show that it is probable that the difficultly oxidizable part of the neutral sulphur occurs as sulpho-acids. An increased elimination of neutral sulphur has been observed in various diseases, such as pneumonia, cystinuria, and especially where the flow of bile into the intestine is prevented.

The total quantity of sulphur in the urine is determined by fusing the solid urinary residue with saltpeter and caustic alkali or sodium peroxide, or by oxida-

[1] Gscheidlen, Pflüger's Arch., **14**; Munk, Virchow's Arch., **69**.

[2] *Ibid.*, **58**, and Zeitschr. f. physiol. Chem., **9**.

[3] Stadthagen, Virchow's Arch., **100**; Lépine, Compt. rend., **91** and **97**; Harnack and Kleine, Zeitschr. f. Biologie, **37**.

[4] Fr. Müller, Berl. klin. Wochenschr., 1887; Reale and Boeri, Maly's Jahresber., **24**; Harnack and Kleine, l. c.; Presch, Virchow's Arch., **119**; Yvon, Arch. de Physiol. (5), **10**.

[5] Benedict, Zeitschr. f. klin. Med., **36**; Harnack and Kleine, l. c.; Folin, Amer. Journ. of Physiol., **13**.

[6] Lépine, l. c.; Smith, Zeitschr. f. physiol. Chem., **17**.

tion with nitric acid.[1] The quantity of neutral sulphur is determined as the difference between the total sulphur and the sulphur of the sulphate and ethereal-sulphuric acids. The readily oxidizable part of the neutral sulphur is determined by oxidation with bromine or potassium chlorate and hydrochloric acid (LÉPINE, JEROME [2]).

Sulphuretted hydrogen occurs in the urine only under abnormal conditions or as a decomposition product. This compound may be produced from the neutral sulphur of the organic substances of the urine by the action of certain bacteria (FR. MÜLLER, SALKOWSKI [3]). Other investigators have given *hyposulphites* as the source of the sulphuretted hydrogen. The occurrence of hyposulphites in normal human urine, which is asserted by HEFFTER, is disputed by SALKOWSKI and PRESCH.[4] Hyposulphites occur constantly in cat's urine and, as a rule, also in dog's urine.

Antoxyproteic acid is a nitrogenous acid containing sulphur which BONDZYNSKI, DOMBROWSKI, and PANEK [5] have isolated from human urine. The composition of the acid was: C 43.21, H 4.91, N 24.4, S 0.61, and O 26.33 per cent. A part of the sulphur can be split off by alkali. This acid is soluble in water, is dextrorotatory, and is only precipitated from concentrated solution by phosphotungstic acid. It does not give the protein color reactions, but gives EHRLICH'S diazo reaction (see below). The salts with the alkalies, barium, calcium, and silver are soluble in water, and of these salts that with barium and, to a still higher degree, the silver salt are soluble with difficulty in alcohol. The free acid and its salts are precipitated by mercuric nitrate and acetate, and by this last reagent even from solutions strongly acidified with acetic acid. Basic lead acetate does not precipitate the pure acid.

Oxyproteic acid is the name given by BONDZYNSKI and GOTTLIEB [6] to a nitrogenous acid containing sulphur and which they prepared from human urine, which has recently been further studied by BONDZYNSKI, DOMBROWSKI and PANEK. This acid contained C 39.62, H 5.64, N 18.08, S 1.12, and O 35.54 per cent, and also contains sulphur which could be split off. On cleavage it yields no tyrosine, nor does it give EHRLICH'S diazo reaction, the xanthoproteic nor the biuret reaction. It gives a faint indication of a MILLON reaction and is not precipitated by phosphotungstic acid, hence it leads to an error in the PFLÜGER-BOHLAND'S method for estimating urea. The acid soluble in water is precipitated by mercuric nitrate and acetate in neutral solutions, but is not precipitated by basic lead acetate. The salts of this acid are readily soluble in water and more soluble in alcohol than the corresponding salts of antoxyproteic acid.

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[1] See Abderhalden and Funk, Zeitschr. f. physiol. Chem., **58** and **59**, which also cites other methods. See Folin, Journ. of biol. Chem., **1**.

[2] Jerome, Pflüger's Arch., **60**.

[3] Fr. Müller, Berlin. klin. Wochenschr., 1887; Salkowski, *ibid.*, 1888.

[4] Heffter, Pflüger's Arch., **38**; Salkowski, *ibid.*, **39**; Presch, Virchow's Arch., **119**.

[5] Zeitschr. f. physiol. Chem., **46**.

[6] Centralbl. f. d. med. Wissensch., 1897, No. 33.

The acid which is found in large quantities, especially in the urine of dogs poisoned with phosphorus (Bondzynski and Gottlieb), is considered like the preceding acid as an intermediary oxidation product of the proteins, and oxyproteic acid seems to represent a higher state of oxidation or a demolition of the proteins than the antoxyproteic acid.

The acid called *uroproteic acid* by Cloetta is probably a mixture of several bodies, according to the recent investigations of Bondzynski, Dombrowski, and Panek. The same applies also to the barium oxyproteate prepared by Pregl [1] from the urine.

Alloxyproteic acid is a third acid related to the above, which was first isolated by Bondzynski and Panek [2] from the urine and then carefully studied with Dombrowski. The composition is: C 41.33, H 5.70, N 13.55, S 2.19, and O 37.23 per cent, based upon new investigations. The free acid is soluble in water. It gives neither the biuret reaction nor Ehrlich's reaction, and is not precipitated by phosphotungstic acid. Differing from the other acids, it is precipitated by basic lead acetate, and its salts are only slightly soluble in alcohol. According to Liebermann [3] this acid is not a unit substance, and contains a part of its sulphur as ethereal sulphuric acid and it also contains uroferric acid.

The preparation of the three above-mentioned acids is based in part upon the fact that alloxyproteic acid alone is precipitated by basic lead acetate and that the two other acids can be precipitated from the filtrate by mercuric acetate, the antoxyproteic acid in acetic acid solution and the oxyproteic acid in neutral solution. The preparation is nevertheless very tedious and complicated and therefore we must refer to the original works [4] for details.

Uroferric acid is an acid isolated by Thiele [5] from the urine, according to Siegfried's method for preparing pure peptone. It also contains sulphur, 3.46 per cent, and has the formula $C_{35}H_{56}N_8SO_{19}$. The acid forms a white powder which is readily soluble in water, saturated ammonium-sulphate solution, and methyl alcohol. It is soluble with difficulty in absolute alcohol, insoluble in benzene, chloroform, ether, and acetic ether. About one half of the sulphur can be split off as sulphuric acid on boiling with hydrochloric acid. The acid gives neither the biuret test nor Millon's or Adamkiewicz's reactions. It is precipitated by mercuric nitrate and sulphate, and also by phosphotungstic acid. This acid is hexabasic, and its specific rotation at 18° C. $(\alpha)_D = -32.5°$. On cleavage it yields melanine substances, sulphuric acid, aspartic acid, but no hexone

[1] Cloetta, Arch. f. exp. Path. u. Pharm., **40**; Pregl, Pflüger's Arch., **75**.
[2] Ber. d. d. chem. Gesellsch., **35**.
[3] Zeitschr. f. physiol. Chem., **52**.
[4] *Ibid.*, **46**.
[5] *Ibid.*, **37**.

bases. The existence of this acid is disputed by Bondzynski, Dombrowski and Panek. The investigations of Ginsberg also contradict the occurrence of such an acid, because no sulphuric acid could be split off from the mixture of the oxyproteic acids by hydrolysis.

Methods for the quantitative estimation of the total oxyproteic acids have been suggested by Ginsberg and by Gawinski [1]. According to their determinations in man with a mixed diet the nitrogen of the oxyproteic acids represented 3–6.8 per cent of the total nitrogen, and with a milk diet it sinks to about one-half of this (Gawinski). In dogs it amounts to 2 per cent of the total nitrogen (Ginsberg). In disease it may rise, and in typhoid cases it may rise to 14.69 per cent of the total nitrogen (Gawinski). In phosphorus poisoning this nitrogen fraction is also markedly increased according to several observations. The oxyproteic acids are considered, as above remarked, as intermediary products of the protein metabolism, and Gawinski holds that the elimination of their nitrogen runs parallel with the elimination of neutral sulphur, so that this latter may serve as an approximate measure of the elimination of these acids.

Abderhalden and Pregl [2] have shown that human urine normally contains compounds which stand, perhaps, in close relation to the polypeptides, and which on hydrolysis with acids yield at least a part of the moities existing in the protein molecule. In the case investigated they obtained abundant glycocoll, also leucine, alanine, glutamic acid, phenylalanine, and probably also aspartic acid. The relation between these polypeptide-like bodies and the above-mentioned proteic acids and uroferric acid has not been investigated.

Non-dialyzable substances, the so-called *adialyzable bodies*, or bodies that dialyze with difficulty, also occur in the urine. They consist in part of chrondroitin-sulphuric acid whose daily amount, according to Pons, is 0.08–0.09 gram, and also of nucleic acid, mucoids and unknown bodies. Sasaki found 0.218–0.68 gram of such bodies per liter of normal urine, and Ebbecke found 1.44 grams in men. In pregnant women Savarè found somewhat higher results (0.6 gram per liter) than in non-pregnant women (0.4 gram). The quantity is increased in fevers, in pneumonia (Ebbecke), in nephritis, and especially in eclampsia, where Savarè [3] indeed in one case found 13.84 grams per liter. The adialyzable bodies occurring in eclampsia are toxic.

Amino-acids may, when they are introduced into the body in large amounts, also pass in part into the urine. This has been shown for *r*-alanine by R. Hirsch in the dog, and by Plaut and Reese in dog and man, and for *r*-leucine by Abderhalden and Samuely [4] in rabbits. Embden and Reese, Forssner, Abderhalden and Schittenhelm, Samuely, Embden and Marx [5] were able,

[1] Gawinski, Zeitschr. f. physiol. Chem., 58. ; Ginsberg, Hofmeister's Beiträge, 10.

[2] Zeitschr. f. physiol. Chem., 46.

[3] Pons, Hofmeister's Beiträge, 9; Sasaki, *ibid.*, 9; Savarè, *ibid.*, 9 and 11; Ebbecke, Bioch. Zeitschr., 13.

[4] R. Hirsch, Zeitschr. f. exp. Path. u. Therap., 1; Plaut and Reese, Hofmeister's Beiträge, 7; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., 47.

[5] Forssner, Zeitschr. f. physiol. Chem., 47; Abderhalden and Schittenhelm, *ibid.*, 47; Samuely, *ibid.*, 47; Embden and Reese, Hofmeister's Beiträge, 7, with Marx,

by means of the naphthalene sulphochloride method to detect glycocoll in normal human urine, and this glycocoll must occur in the urine in a combination which is readily split by alkali. Although there have been numerous investigations, no amino-acids besides glycocoll could be detected in normal human urine, while, on the contrary, in pathological conditions other amino-acids have been found several times. The amino-acid fraction of the urine seems to be increased in starvation and in high altitudes (LOEWY [1]). The conclusions of various investigators [2] in regard to the behavior of amino-acids in diseases such as gout, disagree. For the quantitative estimation of the amino-acids we can, according to HENRIQUES,[3] make use to advantage of the method suggested by SÖRENSEN, mentioned on page 162.

Organic combinations containing phosphorus such as glycerophosphoric acid, phosphocarnic acid (ROCKWOOD), etc., which yield phosphoric acid on fusing with saltpeter and caustic alkali, are also found in urine (LÉPINE and EYMONNET, OERTEL). With a total elimination of about 2.0 grams total P_2O_5, OERTEL found on an average about 0.05 gram P_2O_5 as phosphorus in organic combination. According to SYMMERS [4] the organic combined phosphoric acid may in many pathological conditions be 25–50 per cent of the total phosphoric acid. In lymphatic leucæmia, and especially in degenerative diseases of the nervous system, the quantity may increase.

Enzymes of various kinds have been isolated from the urine. Among these may be mentioned *pepsin* (BRÜCKE and others), which, according to MATTHES, undoubtedly originates from the stomach, and a *diastatic enzyme* (COHNHEIM and others) and *trypsin*.[5]

Mucin. The nubecula consists, as shown by K. MÖRNER,[6] of a mucoid which contains 12.74 per cent N and 2.3 per cent S. This mucoid, which apparently originates in the urinary passages, may pass to a slight extent into solution in the urine. In regard to the nature of the mucins and nucleoalbumins otherwise occurring in the urine we refer the reader to the pathological constituents of the urine.

Ptomaines and *leucomaines*, or poisonous substances of an unknown kind, which are often described as alkaloidal subtances, occur in normal urine, as shown by earlier investigations (POUCHET, BOUCHARD, ADUCCO and others[7]) and also by recent researches of KUTSCHER, LOHMANN and ENGELAND. The *trimethylamine*, which originates from the phosphatides, and first detected by DE FILIPPI and later by K. BAUER belong to the leucomaines and also the bases found by KUTSCHER and by KUTSCHER and LOHMANN, namely, *methyl guanidine* (also found by ACHELIS), *dimethylguanidine*, *novain* (previously found by DOMBROWSKI), *reductonovain* $C_7H_{17}NO_2$, *gynesin*, $C_{19}H_{23}N_3O_3$ (from female urine), *mingin*, $C_{13}H_{18}N_2O_2$, *vitiatin* (Chapter XI) and *methylpyridine chloride*, which is not a

ibid., **11**, which also cites the rather conflicting deductions of Neuberg and Wolgemuth and of Hirschstein.

[1] Deutsch. med. Wochenschr., 1905.

[2] See Jastrowitz, Arch. f. exp. Path. u. Pharm., **59**; Walker Hall, Bioch. Journ., **1**; Brugsch and Schittenhelm, Zeitschr. f. exp. Path. u. Therap., **4**.

[3] Zeitschr. f. physiol. Chem., **60**.

[4] Rockwood, Arch. f. (Anat. u.) Physiol., 1895; Oertel, Zeitschr. f. physiol. Chem. **26**, which cites the other works. See also Keller, Zeitschr, f. physiol. Chem., **29**; Mandel and Oertel, N. Y. Univ. Bull. Med. Sciences, **1**; and Maly's Jahresber., **31**; Symmers, Journ. of Path. and Bact., **10**.

[5] In regard to the literature on enzymes in the urine, see Huppert-Neubauer, 599; Matthes, Arch. f. exp. Path. u. Pharm., **49**; Wilenko, Berl. klin. Wochenschr., **45**.

[6] Skand. Arch. f. Physiol., **6**.

[7] A complete bibliography on the ptomaines and leucomaines of the urine is found in Huppert-Neubauer, 403.

leucomaine, but is probably derived from smoking tobacco or from drinking coffee. The imidazole derivatives *histidine* and *imidazolamino-acetic acid* found by KUTSCHER and ENGELAND [1] also belong to this group.

Under pathological conditions the quantity of lucomaines and other bodies may be increased (BOUCHARD, LÉPINE and GUERIN, VILLIERS, GRIFFITHS, ALBU, and others). Within the last few years the poisonous properties of urine have been the subject of more thorough investigation, especially by BOUCHARD. He found that the night urine is less poisonous than the day urine, and that the poisonous constituents of the day and night urine have not the same action. In order to be able to compare the toxic power of the urine under different conditions, BOUCHARD determines the UROTOXIC COEFFICIENT, which is the weight of rabbit in kilos that is killed by the quantity of urine excreted in twenty-four hours by 1 kilo of the person experimented upon.[2]

BAUMANN and V. UDRÁNSZKY have shown that ptomaines may occur in the urine under pathological conditions. They demonstrated the presence of the two ptomaines discovered and first isolated by BRIEGER—*putrescine*, $C_4H_{12}N_2$ (tetramethylenediamine), and *cadaverine*, $C_5H_{14}N_2$ (pentamethylenediamine)—in the urine of a patient suffering from cystinuria and catarrh of the bladder. Cadaverine has later been found by STADTHAGEN and BRIEGER in the urine in two cases of cystinuria. BRIEGER, V. UDRÁNSZKY and BAUMANN, and STADTHAGEN have shown that neither these nor other diamines occur under physiological conditions, while DOMBROWSKI,[3] on the contrary, found cadaverine in normal urine.

Many substances have been observed in animal urine which are not found in human urine. To these belong the above-described *kynurenic acid*, *urocanic acid*, also found in dog's urine and which seems to stand in some relation to the purine bases; *damaluric acid* and *damolic acid* (according to SCHOTTEN,[4] probably a mixture of benzoic acid with volatile fatty acids), obtained by the distillation of cow's urine; and lastly *lithuric acid*, found in the urinary concrements of certain animals.

III. INORGANIC CONSTITUENTS OF URINE.

Chlorides. The chlorine occurring in the urine is undoubtedly combined with the bases contained in this excretion; the chief part is in combination with sodium. In accordance with this, the quantity of chlorine in the urine is generally expressed as NaCl.

The question as to whether a part of the chlorine contained in the urine exists as organic combinations, as considered by BERLIOZ and LEPINOIS, is still disputed, although recently BAUMGARTEN [5] has supported this view.

The quantity of chlorine combinations in the urine is subject to considerable variation. In general the amount from a healthy adult on a

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[1] de Filippi, Zeitschr. f. physiol. Chem., **49**; Bauer, Hofmeister's Beiträge, **11**; Kutscher, Zeitschr. f. physiol. Chem., **51**, with Lohmann, *ibid.*, **48** and **49**; Achelis, *ibid.*, **50**; Engeland, *ibid.*, **57**, and Münch. med. Wochenschr., **55**.

[2] See footnote 7, page 718.

[3] Baumann and Udránszky, Zeitschr. f. physiol. Chem., **13**; Stadthagen and Brieger, Virchow's Arch., **115**; Dombrowski, Arch. polonais. d. sciences biol., 1903.

[4] Zeitschr. f. physiol. Chem., **7**.

[5] Berlioz and Lepinois, see Chem. Centralbl., 1894, **1**, and 1895, **1**; also Petit and Terrat, *ibid.*, 1894, **2**, and Vitali, *ibid.*, 1897, **2**; Ville and Moitessier, Maly's Jahresber., **31**; Meillère, *ibid.*; Bruno, *ibid.*, 452; Baumgarten, Zeitschr. f. exp. Path. u. Therap., **5**.

mixed diet is10–15 grams of NaCl per twenty-four hours. The quantity of common salt in the urine depends chiefly upon the amount of salt in the food, with which the elimination of chlorine increases and decreases. The free drinking of water also increases the elimination of chlorine, which is greater during activity than during rest (at night). Certain organic chlorine combinations, such as chloroform, may increase the elimination of inorganic chlorides by the urine (ZELLER, KAST [1]).

In diarrhœa, in quick formation of large transudates and exudates, also in specially marked cases of acute febrile diseases at the time of the crisis, the elimination of NaCl is materially decreased. The excretion of chlorine may vary considerably in disease, but still the NaCl taken with the food has here, as in physiological conditions, a great influence on the NaCl excretion.[2]

The *quantitative estimation of chlorine* in the urine is most simply performed by titration with silver-nitrate solution. The urine must not contain either proteid (which if present must be removed by coagulation) or iodine or bromine compounds.

In the presence of bromides or iodides evaporate a measured quantity of the urine to dryness, fuse the residue with saltpeter and soda, dissolve the fused mass in water, and remove the iodine or bromine by the addition of dilute sulphuric acid and some nitrite, and thoroughly shake with carbon disulphide. The liquid thus obtained may now be titrated with silver nitrate according to VOLHARD'S method. The quantity of bromide or iodide is calculated as the difference between the quantity of silver-nitrate solution used for the titration of the solution of the fused mass and the quantity used for the corresponding volume of the original urine.

The otherwise excellent titration method of MOHR, according to which we titrate with silver nitrate in neutral liquids, using neutral potassium chromate as an indicator, cannot be used directly on the urine in careful work. Organic urinary constituents are also precipitated by the silver salt, and the results are therefore somewhat high for the chlorine. If this method is to be employed, the organic urinary constituents must first be destroyed. For this purpose evaporate to dryness 5–10 cc. of the urine, after the addition of 1 gram of chlorine-free soda and 1–2 grams chlorine-free saltpeter, and carefully fuse. The mass is dissolved in water, acidified faintly with nitric acid, and then neutralized exactly with pure calcium carbonate. This neutral solution is used for the titration.

The silver-nitrate solution may be a N/10 one. It is often made of such a strength that each cubic centimeter corresponds to 0.006 gram Cl or 0.01 gram NaCl. This last-mentioned solution contains 29.075 grams of $AgNO_3$ in 1 liter.

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[1] Zeller, Zeitschr. f. physiol. Chem., 8; Kast, *ibid.*, **11**; Vitali, Chem. Centralbl., 1899, 2.

[2] On the elimination of chlorine in disease, see Albu and Neuberg, Physiol. u. Pathol. des Mineralstoffwechsels, Berlin, 1906.

Freund and Toepfer, as well as Bödtker,[1] have suggested modifications of Mohr's method.

Volhard's Method. Instead of the preceding determination, Volhard's method, which can be performed directly on the urine, may be employed. The principle is as follows: All the chlorine from the urine acidified with nitric acid is precipitated by an excess of silver nitrate, filtered, and in a measured part of the filtrate the quantity of silver added in excess is determined by means of a sulphocyanide solution. This excess of silver is completely precipitated by the sulphocyanide, and a solution of some ferric salt, which, as is well known, gives a blood-red reaction with the smallest quantity of sulphocyanide, is used as an indicator.

We require the following solutions for this titration: 1. A silver-nitrate solution which contains 29.075 grams of $AgNO_3$ per liter and of which each cubic centimeter corresponds to 0.01 gram NaCl or 0.00607 gram Cl. 2. A saturated solution at the ordinary temperature of chlorine-free iron alum or ferric sulphate. 3. Chlorine-free nitric acid of a specific gravity of 1.2. 4. A potassium-sulphocyanide solution which contains 8.3 grams KCNS per liter, and of which 2 cc. corresponds to 1 cc. of the silver-nitrate solution.

About 9 grams of potassium sulphocyanide are dissolved in water and diluted to 1 liter. The quantity of KCNS contained in this solution is determined by the silver-nitrate solution in the following way: Measure exactly 10 cc. of the silver solution and treat it with 5 cc. of nitric acid and 1–2 cc. of the ferric-salt solution and dilute with water to about 100 cc. Now the sulphocyanide solution is added from a burette, constantly stirring until a permanent faint-red coloration of the liquid takes place. The quantity of sulphocyanide found in the solution by this means indicates how much it must be diluted to be of the proper strength. Titrate once more with 10 cc. of $AgNO_3$ solution and correct the sulphocyanide solution by the careful addition of water until 20 cc. exactly corresponds to 10 cc. of the silver solution.

The determination of the chlorine in the urine is performed by this method in the following way: Exactly 10 cc. of the urine are placed in a flask which has a mark corresponding to 100 cc. and which is provided with a stopper; 5 cc. of nitric acid are added; dilute with about 50 cc. of water and then allow exactly 20 cc. of the silver-nitrate solution to flow in. Close the flask with the stopper and shake well, remove the stopper and wash it with distilled water into the flask, and fill the flask to the 100-cc. mark with distilled water. Close again with the stopper, carefully mix by shaking, and filter through a dry filter. Measure off 50 cc. of the filtrate by means of a dry pipette, add 3 cc. of ferric-salt solution, and allow the sulphocyanide solution to flow in until the liquid above the precipitate has a permanent red color. The calculation is very simple. For example, if 4.6. cc. of the sulphocyanide solution was necessary to produce the final reaction, then for 100 cc. of the filtrate (=10 cc. urine) 9.2 cc. of this solution are necessary. 9.2 cc. of the sulphocyanide solution corresponds to 4.6 cc. of the silver solution, and since 20−4.6=15.4 cc. of the silver solution was necessary to completely precipitate the chlorine in 10 cc. of the urine, then 10 cc. con-

[1] Freund and Toepfer, see Maly's Jahresber., **22**; Bödtker, Zeitschr. f. physiol. Chem., **20**.

tains 0.154 gram of NaCl. The quantity of sodium chloride in the urine is therefore 1.54 per cent, or 15.4 p. m. If we always use 10 cc. for the determination, and always 20 cc. of $AgNO_3$ solution, and dilute with water, to 100 cc., the quantity of NaCl in 1000 parts of the urine is found by subtracting from 20 the number of cubic centimeters of sulphocyanide (R) required with 50 cc. of the filtrate. The quantity of NaCl p.m. therefore under these circumstances = 20 − R, and the percentage of $NaCl = \frac{20 - R}{10}$.

If it is necessary to destroy the organic urinary constituents before titration, this can be best performed, according to DEHN,[1] by evaporating the urine (10 cc.) to dryness on the water-bath after the addition of a small amount of sodium peroxide, then faintly acidifying with nitric acid and then titrating according to VOLHARD. Incineration is unnecessary.

For the approximate estimation of chlorine in the urine EKEHORN has made use of VOLHARD's titration method by using for the determination a glass tube closed at one end and divided into half cubic-centimeters and called the chlorometer. The reagents necessary are : (*a*) a mixture of 20 cc. silver-nitrate solution (according to VOLHARD), 5 cc. nitric acid and water to 100 cc.; (*b*) 40 cc. sulphocyanide solution and 60 cc. of a ferric alum, chlorine-free and saturated at the ordinary temperature. The silver-nitrate solution, of which each cubic centimeter corresponds to 0.002 gm. NaCl, is equivalent to the iron sulphocyanide solution. First 2 cc. of the urine are placed in the graduated tube and then 0.5 cc. sulphocyanide solution, and the silver-nitrate solution gradually added (shaking the tube closed with a rubber stopper) until the coloration of the sulphocyanide just disappears. 0.5 cc. is subtracted from the silver solution for the 0.5 cc. of the sulphocyanide; the tube is so graduated that the quantity of NaCl in the urine in parts per thousand is read off directly on the tube. The difference between these results and those obtained by VOLHARD's titration method amounts only, according to C. TH. MÖRNER,[2] to 0.25 to at most 0.5 p. m.

The approximate estimation of chlorine in the urine (which must be free from protein) is made by strongly acidifying with nitric acid and then adding to it, drop by drop, a concentrated silver-nitrate solution (1 : 8). In a normal quantity of chlorides the drop sinks to the bottom as a rather compact cheesy lump. In diminished quantity of chlorides the precipitate is less compact and coherent, and in the presence of very little chlorine a fine white precipitate or only a cloudiness or opalescence is obtained.

Phosphates. Phosphoric acid occurs in acid urines partly as dihydrogen, MH_2PO_4, and partly as monohydrogen, M_2HPO_4, phosphates, both of which may be found in acid urines at the same time. OTT [3] found that on an average 60 per cent of the total phosphoric acid was di- and 40 per cent was mono- hydrogen phosphate. This proportion may vary, and in many cases the urine seems to contain only dihydrogen phosphate and sometimes indeed only a small quantity of phosphoric acid. The total quantity of phosphoric acid varies and depends on the character and the quantity of food. The average quantity of P_2O_5 is in round numbers 2.5 grams, with a variation of 1–5 grams per

[1] Zeitschr. f. physiol. Chem., **44**.

[2] Ekehorn, Hygiea, Stockholm, 1906; Mörner, Upsala Läkaref. Förh. (N. F.), **11**.

[3] Zeitschr. f. physiol. Chem., **10**.

day. A small part of the phosphoric acid of the urine originates from the burning of organic compounds, such as nuclein, protagon, and lecithin, within the organism; on exclusive feeding with substances rich in nuclein or pseudonuclein the quantity of phosphates is essentially increased; still it is undecided to what extent the excretion of phosphoric acid is a measure of the absorption and decomposition of these bodies.[1] The greater part originates from the phosphates of the food, and the quantity of phosphoric acid eliminated is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesium phosphates. If the food contains much lime and magnesia, large quantities of earthy phosphates are eliminated by the excrement; and even though the food contains considerable amounts of phosphoric acid in these cases, the quantity excreted by the urine is small. This is especially true of herbivora, in which the kidneys are the chief organs for the excretion of alkali phosphates. In man, according to EHRSTRÖM, the content of lime in the food seems to play no important rôle, as in his experiments about one-half of the phosphoric acid taken as $CaHPO_4$ was absorbed; still the extent of phosphoric-acid excretion through the urine depends in man not only upon the total quantity of phosphoric acid in the food, but also upon the relative amounts of the alkaline earths and the alkali salts of the food. In carnivora, in which phosphate injected subcutaneously is eliminated by the intestine (BERGMANN), the urine is habitually poor in phosphates.[2]

As the extent of the elimination of phosphoric acid is mostly dependent upon the character of the food and the absorption of the phosphates in the intestine, it is apparent that the relation between the nitrogen and phosphoric-acid excretion cannot run parallel. This is in fact so, and, according to EHRSTRÖM, the organism has the power of accumulating large quantities of phosphorus for a relatively long time independent of the condition of the nitrogen balance. With a certain regular food the relation between nitrogen and phosphoric acid in the urine can be kept almost constant. Thus on feeding with an exclusive meat diet, as observed by VOIT[3] in dogs, when the nitrogen and phosphoric acid (P_2O_5) of the food exactly reappeared in the urine and feces, the relation was S.1:1. In starvation, as shown by the compilation of R. TIGERSTEDT,[4] the phosphorized constituents of the body are destroyed to a

[1] See A. Gumlich, Zeitschr. f. physiol. Chem., **18**; Roos, *ibid.*, **21**; Weintraud, Arch. f. (Anat. u.) Physiol., 1895; Milroy and Malcolm, Journ. of Physiol., **23**; Röhmann and Steinitz, Pflüger's Arch., **72**; Loewi, Arch. f. exp. Path. u. Pharm., **44** and **45**.

[2] Ehrström, Skand. Arch. f. Physiol., **14**; Bergmann, Arch. f. exp. Path. u. Pharm., **47**.

[3] Physiologie des allgemeinen Stoffwechsels und der Ernährung in L. Hermann's Handbuch, **6**, Thl. 1, 79.

[4] Skand. Arch. f. Physiol., **16**.

much greater extent than when food very poor in phosphorus is given. In starvation this relation is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this is the bone-tissue. According to PREYSZ, OLSAVSZKY, KLUG, and I. MUNK [1] the elimination of phosphoric acid is considerably increased by intense muscular work.

As the phosphoric acid is in part derived from the nucleins, it would be expected that in those diseases in which the excretion of alloxuric bodies was increased the phosphoric acid would also be augumented. This is not the case, and indeed we have observed cases with an increased elimination of alloxuric bodies with a diminution in the phosphoric-acid excretion. Cases of leucæmia have been observed in which the phosphoric-acid excretion was reduced, although there was a pronounced increase in the number of leucocytes. In these cases there may be a subsequent excretion or retention of phosphoric acid. This last condition also occurs in inflammatory and renal diseases. The earthy phosphates of the urine sometimes have the tendency of precipitating either spontaneously or after warming, and this has been called *phosphaturia*. We are here dealing with a diminished acidity and, it seems, with a diminished excretion of phosphoric acid and an increased elimination of lime, or at least an essentially different relation between the phosphoric acid and the alkaline earths of the urine, as compared with the normal (PANEK IWANOFF, SOETBER and KRIEGER [2]).

Quantitative Estimation of the Total Phosphoric Acid in the Urine. This estimation is most simply performed by titrating with a solution of uranium acetate. The principle of the titration is as follows: A warm solution of phosphates containing free acetic acid gives a whitish-yellow precipitate of uranium phosphate with a solution of a uranium salt. This precipitate is insoluble in acetic acid, but dissolves in mineral acids, and on this account there is always added, in titrating, a certain quantity of sodium-acetate solution. Potassium ferrocyanide is used as the indicator, which does not act on the uranium-phosphate precipitate, but gives a reddish-brown precipitate or coloration in the presence of the smallest amount of soluble uranium salt. The solutions necessary for the titration are: 1. A solution of a uranium salt of which each cubic centimeter corresponds to 0.005 gram P_2O_5 and which contains 20.3 grams of uranium oxide per liter. 20 cc. of this solution corresponds to 0.100 gram P_2O_5. 2. A solution of sodium acetate. 3. A freshly prepared solution of potassium ferrocyanide.

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[1] Preysz, see Maly's Jahresber., **21**; Olsavszky and Klug, Pflüger's Arch., **54**; Munk 3, Arch. f. (Anat. u.) Physiol., 1895.

[2] Panek, see Maly's Jahresber., **30**, 112; Iwanoff, Biochem. Centralbl., **1**, 710; Soetber and Krieger, Deutsch. Arch. f. klin. Med., **72**; Campani, Biochem. Centralbl., **3**, 616; Tobler, Arch. f. exp. Path. u. Pharm., **52**.

The uranium solution is prepared from uranium nitrate or acetate. Dissolve about 35 grams uranium acetate in water, add some acetic acid to facilitate solution, and dilute to 1 liter. The strength of this solution is determined by titrating with a solution of sodium phosphate of known strength (10.085 grams crystallized salt in 1 liter, which corresponds to 0.100 gram P_2O_5 in 50 cc). Proceed in the same way as in the titration of the urine (see below), and correct the solution by diluting with water, and titrate again until 20 cc. of the uranium solution corresponds exactly to 50 cc. of the above phosphate solution.

The sodium-acetate solution should contain 10 grams sodium acetate and 10 grams conc. acetic acid in 100 cc. For each titration 5 cc. of this solution is used with 50 cc. of the urine.

In performing the titration, mix 50 cc. of filtered urine in a beaker with 5 cc. of the sodium acetate, cover the beaker with a watch-glass, and warm over the water-bath. Then allow the uranium solution to flow in from a burette, and when the precipitate does not seem to increase, place a drop of the mixture on a porcelain plate with a drop of the potassium-ferrocyanide solution. If the amount of uranium solution added has not been sufficient, the color will remain pale yellow and more uranium solution must be added; but as soon as the slightest excess of uranium solution has been used the color becomes a faint reddish brown. When this point has been obtained, warm the solution again and add another drop. If the color remains of the same intensity, the titration is ended; but if the color varies, add more uranium solution, drop by drop, until a permanent coloration is obtained after warming, and now repeat the test with another 50 cc. of the urine. The calculation is so simple that it is unnecessary to give an example.

In the above manner one determines the total quantity of phosphoric acid in the urine. If we wish to know the phosphoric acid combined with alkaline earths and with alkalies, we first determine the total phosphoric acid in a portion of the urine and then remove the earthy phosphates in another portion by ammonia. The precipitate is collected on a filter, washed, transferred into a beaker with water, treated with acetic acid, and dissolved by warming. This solution is now diluted to 50 cc. with water, and 5 cc. sodium-acetate solution added, then titrated with uranium solution. The difference between the two determinations gives the quantity of phosphoric acid combined with the alkalies. The results obtained are not quite accurate, as a partial transformation of the monophosphates of the alkaline earths and also calcium diphosphate into triphosphates of the alkaline earths and ammonium phosphate takes place on precipitating with ammonia, and the method gives too high results for the phosphoric acid combined with alkalies and remaining in solution.

Sulphates. The sulphuric acid of the urine originates only to a very small extent from the sulphates of the food. A disproportionately greater part is formed by the burning within the body of the proteins which contain sulphur, and it is chiefly this formation of sulphuric acid from the proteins which gives rise to the previously mentioned excess of acids over the bases in the urine. The quantity of sulphuric acid eliminated by the urine amounts to about 2.5 grams H_2SO_4 per day.

As the sulphuric acid chiefly originates from the proteins, it follows that the elimination of sulphuric acid and the elimination of nitrogen runs almost parallel, and the relation $N:H_2SO_4$ is about 5:1. A complete parallelism can hardly be expected, as in the first place a part of the sulphur is always eliminated as neutral sulphur, and secondly because the small proportion of sulphur in different protein bodies undergoes greater variation as compared with the large proportion of nitrogen contained therein. In general the elimination of nitrogen and sulphuric acid under normal and under diseased conditions seems to run parallel. Sulphuric acid occurs in the urine partly preformed (sulphate-sulphuric acid) and partly as ethereal-sulphuric acid. The first is designated as *A*- and the other as *B*-sulphuric acid.

The *quantity of total sulphuric acid* is determined in the following way, but at the same time the precautions described in other works must be observed. 100 cc. of filtered urine is treated with 5 cc. of concentrated hydrochloric acid and boiled for fifteen minutes. While boiling precipitate with 2 cc. of a saturated $BaCl_2$ solution, and warm for a little while until the barium sulphate has completely settled. The precipitate must then be washed with water and also with alcohol and ether (to remove resinous substances), and then treated according to the usual method.

The separate determination of the sulphate-sulphuric acid and the ethereal-sulphuric acid may be accomplished, according to Baumann's method, by first precipitating the sulphate-sulphuric acid by $BaCl_2$ from the urine acidified with acetic acid, then decomposing the ethereal-sulphuric acid by boiling after the addition of hydrochloric acid, and finally determining the sulphuric acid set free as barium sulphate. A still better method is the following, suggested by Salkowski[1]:

200 cc. of urine are precipitated by an equal volume of a barium solution which consists of 2 vols. barium hydrate and 1 vol. barium-chloride solution, both saturated at the ordinary temperature. Filter through a dry filter, measure off 100 cc. of the filtrate which contains only the ethereal-sulphuric acid, treat with 10 cc. of hydrochloric acid of a specific gravity 1.12, boil for fifteen minutes, and then warm on the water-bath until the precipitate has completely settled and the supernatant liquid is entirely clear. Filter and wash with warm water and with alcohol and ether, and proceed according to the generally prescribed method. The difference between the ethereal-sulphuric acid found and the total quantity of sulphuric acid as determined in a special portion of urine is taken to be the quantity of sulphate-sulphuric acid.

Folin[2] has suggested a method for estimating the sulphate-sulphuric acid as well as the ethereal-sulphuric acid, and also the total sulphur, which is unlike the ordinary methods.

Nitrates occur in small quantities in human urine (Schönbein), and they probably originate from the drinking-water and the food. According to Weyl

[1] Baumann, Zeitschr. f. physiol. Chem., 1; Salkowski, Virchow's Arch., 79.

[2] Journ. of Biol. Chem., 1, and Amer. Journ. of Physiol., 13.

and CITRON,[1] the quantity of nitrates is smallest with a meat diet and greatest with vegetable food. The average amount is about 42.5 milligrams per liter.

Potassium and Sodium. The quantity of these bodies eliminated by the urine by a healthy adult on a mixed diet is, according to SALKOWSKI,[2] 3–4 grams K_2O and 5–6 grams Na_2O, with an average of about 2–3 grams K_2O and 4–6 grams Na_2O. The proportion of K to Na is ordinarily 3:5. The quantity depends above all upon the food. In starvation the urine may become richer in potassium than in sodium, which results from the lack of common salt and the destruction of tissue rich in potassium. The quantity of potassium may be relatively increased during fever, while after the crisis the reverse is the case.

The quantitative estimation of these bodies is made by the gravimetric methods as described in works on quantitative analysis. In the determination of the total alkalies new methods have been devised by PRIBRAM and GREGOR, and for the potassium alone a method by AUTENRIETH and BERNHEIM.[3]

Ammonia. Some ammonia is habitually found in human urine and in that of carnivora. As above stated (page 694), on the formation of urea from ammonia, this quantity may represent the small amount of ammonia which is excluded from the synthesis to urea by being combined with acids formed in excess by combustion and not united with the fixed alkalies. This view is confirmed by the observations of CORANDA, who found that the elimination of ammonia was smaller on a vegetable diet and larger on a rich meat diet than on a mixed diet. On a mixed diet the average amount of ammonia eliminated by the urine is about 0.7 gram NH_3 per day (NEUBAUER), corresponding to 4.6–5.6 per cent of the total nitrogen of the urine according to CAMERER, Jr. As above stated, all the ammonia of the urine is not represented by the residue which has eluded synthesis into urea by neutralization with acids, because, as shown by STADELMANN and BECKMANN,[4] ammonia is eliminated by the urine even during the continuous administration of fixed alkalies.

Ammonia exists on an average of about 0.90 milligram in 100 cc. of human blood, and in different amounts in all the tissues thus far investigated.[5] According to NENCKI and ZALESKI [6] it is abundantly formed in

[1] Schönbein, Journ. f. prakt. Chem., **92**; Weyl, Virchow's Arch., **96**, with Citron, *ibid.*, **101**.

[2] *Ibid.*, **53**.

[3] Pribram and Gregor, Zeitschr. f. analyt. Chem., **38**; Autenreith and Bernheim, Zeitschr. f. physiol. Chem., **37**.

[4] Coranda, Arch. f. exp. Path. u. Pharm., **12**; Stadelmann (and Beckmann), Einfluss der Alkalien auf den Stoffwechsel, etc. Stuttgart, 1890; Camerer, Zeitschr. f. Biologie, **43**.

[5] See Salaskin, Zeitschr. f. physiol. Chem., **25**, 449, and footnote 6, page 317.

[6] Arch. des science biol. de St. Pétersbourg, **4**, and Salaskin, l. c. See also Nencki and Zaleski, Arch. f. exp. Path. u. Pharm., **37**.

the cells of the digestive glands, the stomach, the pancreas, and the intestinal mucosa (of dogs) as the time when protein foods are being digested and transported to the liver. As the ammonia introduced into the liver is transformed into urea (see above), we can therefore expect that in certain diseases of the liver an increased elimination of ammonia and a decreased excretion of urea will occur. In how far this is true has already been stated (page 651), and we refer to the researches of the various authors there cited.

In man and certain animals the elimination of ammonia is increased by the introduction of mineral acids; and, as shown by JOLIN,[1] organic acids, such as benzoic acid, which are not destroyed in the body act in a similar manner. The ammonia set free in the protein destruction is in part used in the neutralization of the acids introduced, and in this way a destructive removal of fixed alkalies is prevented. This dissimilar tendency of different animals toward acidosis has been discussed in the previous pages.

Acids formed in the destruction of proteins in the body act on the elimination of ammonia like those introduced from without. For this reason the quantity of ammonia in human urine is increased under such conditions and in such diseases where an increased formation of acid takes place because of an increased metabolism of proteins. This is the case with a lack of oxygen in fevers and diabetes. In the last-mentioned disease, organic acids—β-oxybutyric acid and acetoacetic acid—are produced, which pass into the urine combined with ammonia.[2] Other observations also indicate that the elimination of ammonia by the urine is increased on insufficient or diminished supply of alkalies or alkaline earths.

The detection and quantitative estimation of ammonia used to be performed according to the method suggested by SCHLÖSING. The principle of this method is that the ammonia from a measured amount of urine is set free by lime-water in a closed vessel and absorbed by a measured amount of N/10 sulphuric acid. After the absorption of the ammonia the quantity is determined by titrating the remaining free sulphuric acid with a N/10 caustic-alkali solution. This method gives low results, and in exact work we must proceed as suggested by BOHLAND.[3]

The recent methods for estimating the ammonia are all based upon the distillation of the ammonia, after the addition of lime, magnesia, or alkali carbonate, at low temperatures either by the aid of vacuum (NENCKI and ZALESKI, WURSTER, KRÜGER, REICH and SCHITTENHELM,

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[1] Jolin, Skand. Arch. f. Physiol., **1**. In regard to the behavior of ammonium salts in the animal body, see Rumpf and Kleine, Zeitschr. f. Biologie, **34**; Kowalewski and Markewicz, Bioch. Zeitschr., **4**, and the works cited on page 641.

[2] On the elimination of ammonia in disease, see the works of Rumpf, Virchow's Arch., **143**; Hallervorden, *ibid.*

[3] Pflüger's Arch., **43**, 32.

and SCHAFFER) or by the aid of a current of air (FOLIN) and then collecting it in a standard acid.

According to the methods suggested by KRÜGER, REICH and SCHITTENHELM [1] 25 cc. of the urine are placed in a distillation-flask with about 10 grams of NaCl and 1 gram of Na_2CO_3, and this distilled at 43° C. and a pressure of 30–40 millimeters Hg with the aid of an air-pump. Alcohol is added to prevent foaming. The ammonia is absorbed in N/10 acid contained in a PELIGOT tube surrounded by ice-water, and when the distillation is finished the acid is retitrated, making use of rosolic acid as indicator. In regard to details, see the original publications. SCHAFFER'S method is practically the same STEEL and GIES have raised objections to FOLIN'S method (passing air through the urine), as they found low results in the presence of large quantities of magnesium phosphate in the urine. MALFATTI [2] has recently suggested a method based upon an entirely different principle.

Calcium and magnesium occur in the urine chiefly as phosphates. The quantity of earthy phosphates eliminated daily is somewhat more than 1 gram, and of this amount $\frac{2}{3}$ is magnesium and $\frac{1}{3}$ calcium phosphate. This statement, as found by RENWALL and GROSS,[3] is not correct, or at least is not valid in general, as they found more calcium than magnesium in the urine. In acid urines the mono- as well as the dihydrogen earthy phosphates are found, and the solubility of the first, among which the calcium salt $CaHPO_4$ is especially insoluble, is particularly augmented by the presence in the urine of dihydrogen alkali phosphates and sodium chloride (OTT [4]). The quantity of alkaline earths in the urine depends on the composition of the food. The lime-salts absorbed are in great part excreted again into the intestine, and the quantity of lime-salts in the urine is therefore no measure of their absorption. The introduction of readily soluble lime-salts or the addition of hydrochloric acid to the food may therefore cause an increase in the quantity of lime in the urine, while the reverse takes place on adding alkali phosphate to the food. According to GRANSTRÖM [5] starvation in rabbits or the introduction of food which yields an acid ash and yields an acid urine produces the same effect as the introduction of acid. Nothing is known with certainty in regard to the constant and regular change in the elimination of calcium and magnesium salts in disease, and in these conditions the excretion is chiefly dependent upon the diet and the formation and introduction of acid.[6]

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[1] Zeitschr. f. physiol. Chem., **39**; Schaffer, Amer. Journ. of Physiol., 8, which contains the literature.

[2] Steel and Gies, Journ of biol. Chem., **5**; Malfatti, Zeitschr. f. anal. Chem., **47**.

[3] Renwall, Skand. Arch. f. Physiol., **16**; Gross, Biochem. Centralbl., **4**, 189.

[4] Zeitschr. f. physiol. Chem., **10**.

[5] *Ibid.*, **58**.

[6] See Albu and Neuberg, l. c., and Zak, Wien. klin. Wochenschr., **21**.

The quantity of calcium and magnesium is determined according to the ordinary well-known methods.

Iron occurs in the urine only in small quantities, and, as it seems from the investigations of KUNKEL, GIACOSA, KOBERT and his pupils, it does not exist as a salt, but as an organic combination—in part as pigment or chromogen. The reports in regard to the iron present seem to show that the quantity ranges from 1 to 11 milligrams per liter of urine (MAGNIER, GOTTLIEB, KOBERT and his pupils). JOLLES found as an average for twelve persons 8 milligrams of iron in twenty-four hours, while HOFFMANN, NEUMANN and MAYER[1] found lower results—an average of 1.09 and 0.983 milligrams. The quantity of *silicic acid* is ordinarily stated to amount to about 0.3 p. m. Traces of *hydrogen peroxide* also occur in the urine.

The *gases* of the urine are carbon dioxide, nitrogen, and traces of oxygen. The quantity of nitrogen is not quite 1 vol. per cent. The carbon dioxide varies considerably. In acid urines it is hardly one-half as great as in neutral or alkaline urines.

IV. THE QUANTITY AND QUANTITATIVE COMPOSITION OF URINE.

The quantity and composition of urine are liable to great variation. The circumstances which under physiological conditions exercise a great influence are the following: the blood-pressure, and the rapidity of the blood-current in the glomeruli. The quantity of urinary constituents, especially water in the blood; and, lastly, the condition of the secretory glandular elements. Above all, the quantity and concentration of the urine depend on the quantity of water which is introduced into the blood or which leaves the body in other ways. The excretion of urine is increased by drinking freely or by reducing the quantity of water otherwise removed; and it is decreased by a diminished ingestion of water or by a greater loss of water in other ways. Ordinarily in man just as much water is eliminated by the kidneys as by the skin, lungs, and intestine together. At lower temperatures and in moist air, since under these conditions the elimination of water by the skin is diminished, the excretion of urine may be considerably increased. Diminished introduction of water or increased elimination of water by other means—as in violent diarrhœa or vomiting, or in profuse perspiration—greatly diminishes the amount of urine excreted. For example, the urine may sink as low as 500–400 cc. per day in intense summer heat, while after copious draughts of water the elimination of 3000 cc. of urine has been observed during the same time. The quantity of urine voided in the course of twenty-four hours varies considerably from day to day, the average being ordinarily cal-

[1] Kunkel, cited from Maly's Jahresber., **11**; Giacosa, *ibid.*, **16**; Kobert, Arbeiten des Pharm. Inst. zu Dorpat, **7**; Magnier, Ber. d. deutsch. chem. Gesellsch., **7**; Gottlieb, Arch. f. exp. Path. u. Pharm., **26**; Jolles, Zeitschr. f. anal. Chem., **36**; Hoffmann, Zeitschr. f. anal. Chem., **40**; Neumann and Mayer, Zeitschr. f. physiol. Chem., **37**.

culated as 1500 cc. for healthy adult men and 1200 cc. for women. The minimum elimination occurs during the early morning between 2 and 4 o'clock; the maximum, in the first hours after waking and from 1–2 hours after a meal.

The quantity of solids excreted per day is nearly constant, even though the quantity of urine may vary, and it is quite constant when the manner of living is regular. Therefore the percentage of solids in the urine is naturally in inverse proportion to the quantity of urine. The average amount of solids per twenty-four hours is calculated as 60 grams. The quantity may be calculated with approximate accuracy from the specific gravity if the second and third decimals of this factor be multiplied by HÄSER'S coefficient, 2.33. The product gives the amount of solids in 1000 cc. of urine, and if the quantity of urine eliminated in twenty-four hours be measured, the quantity of solids in twenty-four hours may be easily calculated. For example, 1050 cc. of urine of a specific gravity 1.021 was eliminated in twenty-four hours; therefore the quantity of solids excreted was $21 \times 2.33 = 48.9$ and $\frac{48.9 \times 1050}{1000} = 51.35$ grams. LONG [1] has made a new determination of the coefficient for a specific gravity taken at 25° C. and finds that it is equal to 2.6, which almost corresponds to HÄSER'S coefficient at 15° C.

Those bodies which, under physiological conditions, affect the density of the urine are common salt and urea. The specific gravity of the first is 2.15 and the last only 1.32, so it is easy to understand, when the relative proportion of these two bodies essentially deviates from the normal, why the above calculation from the specific gravity is not exact. The same is true when a urine poor in normal constituents contains large amounts of foreign bodies, such as albumin or sugar.

As above stated, the percentage of solids in the urine generally decreases with a greater elimination, and a very considerable excretion of urine (*polyuria*) has therefore, as a rule, a lower specific gravity. An important exception to this rule is observed in urine containing sugar (*diabetes mellitus*), in which there is a copious excretion with a very high specific gravity due to the sugar. In cases where very little urine is excreted (*oliguria*), e.g., during profuse perspiration, in diarrhœa, and in fevers, the specific gravity of the urine is as a rule very high; the percentage of solids is also high and the urine has a dark color. Sometimes, as for example in certain cases of albuminuria, the urine may have a low specific gravity notwithstanding the oliguria, and be poor in solids and light in color.

In certain cases it is interesting to know the relation between the carbon and the nitrogen, or the quotient C/N. This factor may vary between 0.7 and 1; as a rule, it amounts on an average to 0.87, but changes

[1] Journ. Amer. Chem. Soc., 25.

according to the nature of the food and is higher after a diet rich in carbohydrates than after food rich in fat (PREGL, TANGL, LANGSTEIN and STEINITZ, and others [1]).

It is difficult to give a tabular view of the composition of urine on account of its variation. For certain purposes the following table may be of some value, but it must not be overlooked that the results are not given for 1000 parts of urine, but only approximate figures for the quantities of the most important constituents which are eliminated during the course of twenty-four hours in a volume of 1500 cc. of urine. These figures apply only to a diet which corresponds to VOIT's standard figures, namely 118 grams protein, 56 grams fat, and 500 grams carbodydrate per day, and to a man of average weight.

Daily quantity of solids = 60 grams.

Organic constituents = 35 grams.		Inorganic constituents = 25 grams.	
Urea	30.0 grams.	Sodium chloride (NaCl)	15.0 grams.
Uric acid	0.7 "	Sulphuric acid (H_2SO_4)	2.5 "
Creatinine	1.5 "	Phosphoric acid (P_2O_5)	2.5 "
Hippuric acid	0.7 "	Potash (K_2O)	3.3 "
Remaining organic bodies	2.1 "	Ammonia (NH_3)	0.7 "
		Magnesia (MgO) / Lime (CaO)	0.8 "
		Remaining inorganic bodies	0.2 "

Urine contains on an average 40 p. m. solids. The quantity ot urea is about 20 p. m., and common salt about 10 p. m.

The physico-chemical methods are being used in urinary analysis even to a greater extent than in the analysis of other animal fluids. A great number of cryoscopic determinations, but fewer conductivity determinations, have been made. A constant relation between the values found by physico-chemical methods and the analytical methods has been sought, for example, between the freezing-point depression and the specific gravity of the common salt and others; or attempts have been made to find certain constants in the composition of the urine based upon the results of various methods, and in this way to obtain an explanation as to the mechanism of the excretion of urine in order to apply them for diagnostic purposes. The results obtained are, as is to be expected, so variable and dependent upon so many conditions which cannot be controlled that definite conclusions must be drawn with the greatest caution. In regard to the value and usefulness of the various constants and relations which are based upon theoretical considerations, the views are unfortunately still too divergent.

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[1] Pregl, Pflüger's Arch., **75**, which contains the earlier literature. Tangl, Arch. f. (Anat. u.) Physiol., 1899, Suppl.; Langstein and Steinitz, Centralbl. f. Physiol., **19**.

V. CASUAL URINARY CONSTITUENTS.

The casual appearance, in the urine, of medicinal agents or of urinary constituents resulting from the introduction of foreign substances into the organism is of practical importance, because such compounds may interfere in certain urinary investigations; they also afford a good means of determining whether certain substances have been introduced into the organism or not. From this point of view a few of these bodies will be spoken of in a following section (on the pathological urinary constituents). The presence of these foreign bodies, in the urine, is of special interest in those cases in which they serve to elucidate the chemical transformations which certain substances undergo within the organism. As inorganic substances generally leave the body unchanged,[1] they are of very little interest from this standpoint; but the changes which certain organic substances undergo when introduced into the animal body may be studied by the transformation products as found in the urine.

The bodies belonging to the fatty series undergo, though not without exceptions, a combustion leading toward the final products of metabolism; still, often a greater or smaller part of the bodies in question escape oxidation and appear unchanged in the urine. A part of the acids belonging to this series, which are otherwise decomposed into water and carbonates and render the urine neutral or alkaline, may act in this manner. The *volatile fatty acids* poor in carbon are less easily oxidized than those rich in carbon, and they therefore pass unchanged into the urine in large amounts. This is especially true of formic and acetic acids (Schotten, Gréhant and Quinquaud [2]), but as to *oxalic acid* authorities disagree. In birds, according to Gaglio and Giunti, it is not oxidized. In mammals it is in great part oxidized, according to Giunti, while Gaglio and Pohl claim that it is not destroyed. Marfori and Giunti claim that in human beings oxalic acid is in great part oxidized. The recent investigations of Salkowski, Pierallini, Stradomsky, Klemperer and Tritschler and especially those of Hildebrandt and of Dakin,[3] where oxalates were introduced subcutaneously, show that oxalic acid, when introduced in medium quantities, is in great part oxidized in the animal body. *Tartaric acids* act differently, according

[1] In regard to the behavior of certain of these bodies, see Heffter, Die Ausscheidung körperfremden Substanzen im Harn, Ergebnisse d. Physiol., **2**, Abt. 1.

[2] Schotten, Zeitschr. f. physiol. Chem., **7**; Gréhant and Quinquaud, Compt. rend., **104**.

[3] Gaglio, Arch. f. exp. Path. u. Pharm., **22**; Giunti, Chem. Centralbl., 1897, **2**; Marfori, Maly's Jahresber., **20** and **27**; Pohl, Arch. f. exp. Path. u. Pharm., **37**; Salkowski, Berl. klin. Wochenschr., 1900; Pierallini, Virchow's Arch., **160**; Stradomsky, *ibid.*, **163**; Klemperer and Tritschler, Zeitschr. f. klin. Med., **44**; Hildebrandt, Zeitschr. f. physiol. Chem., **35**; Dakin, Journ. of biol. Chem., **3**.

to BRION [1]; thus in dogs the levotartaric acid is almost entirely consumed, while a little more than 70 per cent of dextrotartaric acid is burnt. *Racemic acid* is oxidized to a still less extent in the animal body. *Succinic* and *malic acids* are completely combustible, according to POHL.[2] Examples of the different behavior of stereoisomeric substances have already been given on page 199.

The *acid amides* do not appear to be altered in the body (SCHULTZEN and NENCKI [3]). The *amino-acids* may, indeed, when introduced into the body in large quantities, be in part eliminated unchanged by the urine; but otherwise, as stated above (page 648) for *leucine*, *glycocoll*, and *aspartic acid*, they are decomposed within the body, and may therefore cause an increased excretion of urea. That in the demolition of the amino-acids a deamidation takes place is shown by alanine yielding lactic acid and diaminopropionic acid, yielding glyceric acid, as mentioned in a previous chapter (VIII). The amino-acids give an instructive example of the unequal behavior of stereometric substances in the animal body, as the racemic acids are so changed and transformed that the component foreign to the body is burnt with more or less difficulty, while the component occurring in the body protein is burnt, on the contrary, with ease and more completely. ABDERHALDEN [4] and his collaborators have shown that the *polypeptides* when introduced into the body are decomposed with the formation of urea similar to the amino-acids, and this probably occurs by a previous decomposition into their simplest fractions, the amino-acids.

We do not know exactly how the amino-acids are split in the animal body. DAKIN has shown that in the oxidation of the amino-acids by hydrogen peroxide outside of the body under certain conditions the reaction proceeds with the formation of ammonia, carbon dioxide and an aldehyde, which latter is then further oxidized with the formation of the corresponding acid and other products. For example, leucine first yielded isovaleric aldehyde, then isovaleric acid, $(CH_3)_2.CH.CH_2.COOH$, from which, DAKIN claims, acetone is derived (see under acetone bodies, page 774), with β-oxyisovaleric acid, $(CH_3)_2.C(OH).CH_2.COOH$, probably as intermediary step. In a series of investigations DAKIN [5] calls attention to the correspondence which exists between the artificial oxidations and several known decompositions in the animal body, which indicate a similar decomposition of amino-acids in the living organism. According to NEUBAUER [6] the destruction of the amino-acids occurs, in that the corresponding keto acid, R.CO.COOH is first formed by an oxidative deamidization, and not, as generally admitted, the corresponding oxyacid. The keto acids are by oxidation with the splitting off of CO_2 then transformed into the next lower fatty acid.

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[1] Zeitschr. f. physiol. Chem., **25**.

[2] Pohl, Arch. f. exp. Path. u. Pharm., **37**, which also contains reports on the intermediary products formed in the oxidation of the fatty bodies.

[3] Zeitschr. f. Biol., 8.

[4] Zeitschr. f. physiol. Chem., **39**, **46**, **47**, **48**, **52**, **55**, **57**.

[5] Dakin's work can be found in Journ. of biol. Chem., **1**, **2**, **3**, **4**.

[6] Cited in Physiol. Centralbl., **23**, 76.

By substituting one of the hydrogen atoms in the NH_2 group of normal α-amino-acids by an alkyl radical (methyl) the combustion of the acids of the series C_2 and C_4 is considerably retarded and almost entirely prevented in the members of the C_5 and C_6 series (FRIEDMANN). *Sarcosine* (methyl glycocoll), $(CH_3)NH.CH_2.COOH$, is not readily burnt, and therefore passes in great part unchanged into the urine, but perhaps also passes in small part into the corresponding uramino-acid, *methylhydantoic acid*, $NH_2.CO.N(CH_3).CH_2.COOH$ (SCHULTZEN[1]), is an example of this kind. The substitution of both hydrogen atoms of the NH_2 group by methyl radicals does not seem to make the demolition of the amino-acids more difficult (FRIEDMANN). The α-amino-acids with branched carbon chains behave differently in the body from the *n*-amino-acids, and the formation of β-oxybutyric acid and acetoacetic acid, which will be discussed on page 775, is an example. No general rule for the mode of demolition of the amino-acids can be given (FRIEDMANN[2]).

The *nitriles*, including hydrocyanic acid, pass, according to LANG, into sulphocyanide combinations, and this sulphocyanide apparently originates from the non-oxidized sulphur of the proteins, which is readily split off. PASCHELES' observations indicate that, in an alkaline reaction and at the temperature of the body, this sulphur can readily convert the alkali-cyanides into sulphocyanides. The alkali sulphocyanides when ingested are almost quantitatively eliminated in the urine, according to POLLAK.[3]

By *substitution with halogens*, bodies otherwise readily oxidizable are converted into difficultly oxidizable ones. While the aldehydes are readily and completely burnt like the primary and secondary alcohols of the fatty series, the halogen substituted alhedydes and alcohols are, on the contrary, difficultly oxidizable. The halogen substitution products of methane (chloroform, iodoform, and bromoform) are at least in part destroyed and the corresponding alkali compounds of the halogen pass into the urine.[4]

By *conjugation with sulphuric acid*, the alcohols which are otherwise readily oxidizable may be guarded against combustion, and consequently the alkali salt of ethylsulphuric acid is not burnt in the body (SALKOWSKI[5]).

The *organic combinations containing sulphur* act differently. *Taurine*,

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[1] Ber. d. deutsch. chem. Gesellsch., **5**. See also Baumann and v. Mering, *ibid.*, **8**, 584, and E. Salkowski, Zeitschr. f. physiol. Chem., **4**, 107.

[2] Hofmeister's Beiträge, **11**.

[3] Lang, Arch. f. exp. Path. u. Pharm., **34**; Pascheles, *ibid.*; Pollak, Hofmeister's Beiträge, **2**.

[4] See Harnack and Gründler, Berlin. klin. Wochenschr., 1883; Zeller, Zeitschr. f physiol. Chem., **8**; Kast, *ibid.*, **11**; Binz, Arch. f. exp. Path. u. Pharm., **28**; Zeehuisen, Maly's Jahresber., **23**.

[5] Pflüger's Arch., **4**.

whose behavior varies in different animals (SALKOWSKI [1]), passes in human beings, at least in part, into the corresponding uramino-acid, *taurocarbamic acid*, $NH_2.CO.NH.C_2H_4.SO_2.OH$. A part of the taurine also appears as such in the urine. In rabbits, when taurine is introduced into the stomach, nearly all its sulphur appears in the urine as sulphuric and *hyposulphurous* acids. After subcutaneous injection the taurine appears again in great part unchanged in the urine. In dogs a great part of the sulphur of *cystine* appears in the urine as sulphate (also as thiosulphate) (BLUM, ABDERHALDEN and SAMUELY [2]).

According to W. SMITH the sulphur of the thio-acids, like thioglycolic acid, $CH_2.SH.COOH$, is in part oxidized to sulphuric acid, and according to GOLDMANN the same result occurs with aminothiolactic acid (cysteine) and the sulphur of the thio-alcohols (ethyl mercaptans). On the contrary, ethylsulphide, sulphonic and sulpho acids in general (SALKOWSKI, SMITH [3]) are not changed into sulphuric acid. Oxyethylsulphonic acid, $HO.C_2H_4.SO_2.OH$, which is in part oxidized to sulphuric acid, is an exception (SALKOWSKI).

Conjugation with glucuronic acid occurs, according to the investigations of SUNDVIK and especially of O. NEUBAUER, in many substituted as well as non-substituted alcohols, aldehydes, and ketones. *Chloral hydrate*, $C_2Cl_3OH + H_2O$, passes, after it has been converted into trichlorethyl-alcohol by a reduction, into a levogyrate reducing acid, *urochloralic acid* or trichlorethylglucuronic acid, $C_2Cl_3H_2.C_6H_9O_7$ (MUSCULUS and v. MERING). Of the primary alcohols investigated by NEUBAUER [4] (upon rabbits and dogs) methyl alcohol gave no conjugated glucuronic acid, and ethyl alcohol only a small amount. Isobutyl alcohol and active amyl alcohol yielded relatively large quantities. Secondary alcohols produced conjugated glucuronic acids, and indeed to a greater extent than the primary alcohols, especially in rabbits. The ketones are reduced in part into secondary alcohols and are partly excreted as the conjugated acid. This could be shown for acetone with rabbits but not with dogs.

The **homo-** and **heterocyclic compounds** pass, as far as is known, into the urine as such, or, after a previous partial oxidation or synthesis with other bodies, and appear as so-called aromatic compounds. That the benzene ring is destroyed in the body in certain cases is very probable.

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[1] Ber. d. deutsch. chem. Gesellsch., **6**, and Virchow's Arch., **58**.

[2] Blum, Hofmeister's Beiträge, **5**; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., **46**.

[3] Smith, Pflüger's Arch., **53**, **55**, **57**, and Zeitschr. f. physiol. Chem., **17**; Salkowski, Virchow's Arch., **66**; Pflüger's Arch., **39**; Goldmann, Zeitschr. f. physiol. Chem., **9**; also Baumann and Kast, *ibid.*, **14**.

[4] Sundvik, Maly's Jahresber., **16**; Musculus and v. Mering, Ber. d. deutsch. chem. Gesellsch., **8**; also v. Mering, *ibid.*, **15**; Zeitschr. f. physiol. Chem., **6**; Külz, Pflüger's Arch., **28** and **33**; O. Neubauer, Arch. f. exp. Path. u. Pharm., **46**.

The fact that benzene may be oxidized outside of the body into carbon dioxide, oxalic acid, and volatile fatty acids has been known for a long time; and as in these cases a rupture of the benzene ring must take place, so also, it must be admitted, when aromatic substances undergo a combustion in the animal body, a splitting of the benzene nucleus with the formation of fatty bodies must be the result. If this does not occur, then the benzene nucleus is eliminated with the urine as an aromatic compound of one kind or another. As the benzene nucleus can protect a substance belonging to the fatty series from destruction when conjugated with it, which is the case with the glycocoll of hippuric acid, it seems that the aromatic nucleus itself may likewise be protected from oxidation in the organism by synthesis with other bodies. The aromatic ethereal-sulphuric acids are examples of this kind.

The difficulty in deciding whether the benzene ring itself is destroyed in the body lies in the fact that we do not know all the different aromatic transformation products which may be produced by the introduction of any such substance into the organism, and which must be sought for in the urine. On this account it is also impossible to learn by exact quantitative determinations whether or not an aromatic substance ingested or absorbed appears again unchanged in the urine. Certain observations render it probable that the benzene ring, as above mentioned, is at least in certain cases destroyed in the body. SCHOTTEN, BAUMANN, and others have found that certain amino-acids, such as *phenylamino-propionic acid, amino-cinnamic acid,* and *tyrosine,* when introduced into the body cause no increase in the quantity of known aromatic substances in the urine; this makes a destruction of these amino-acids in the animal body seem probable. According to F. KNOOP[1] phenyl-α-lactic acid and phenyl-α-ketopropionic acid (phenyl pyroracemic acid) have a similar behavior. According to JUVALTA and PORCHER *phthalic acid* is also burnt in the animal body. The last investigator found that the three phthalic acids have varying effects, as the *o*-acid is almost completely burnt in dogs, while about 75 per cent of the *m*- and *p*- acids are excreted unconsumed. This corresponds with the rule found by R. COHN,[2] that among the di-derivatives of benzene the ortho-compounds are more readily destroyed than the corresponding meta- and para-compounds. The claims of JUVALTA and PORCHER are unfortunately disputed by PRIBRAM and POHL.[3]

[1] Schotten, Zeitschr. f. physiol. Chem., **7** and **8**; Baumann, *ibid.*, **10**, 130. In regard to the behavior of tyrosine, see especially Blendermann, *ibid.*, **6**; Schotten, *ibid.*, **7**; Bass, *ibid.*, **11**; and R. Cohn, *ibid.*, **14**; F. Knoop, Der Abbau aromatischer Fettsäuren im Tierkörper, Habilit.-Schrift, Freiburg, 1904.

[2] Zeitschr. f. physiol. Chem., **17**.

[3] Juvalta, Zeitschr. f. physiol. Chem., **13**; Pribram, Arch. f. exp. Path. u. Pharm., **51**; Porcher, Bioch. Zeitschr., **14**; Pohl, *ibid.*, **16**.

An *oxidation* in the side chain of aromatic compounds is often found, and may also occur in the nucleus itself. As an example, benzene is first oxidized to oxybenzene (SCHULTZEN and NAUNYN), and this is then further in part oxidized into *dioxybenzenes* (BAUMANN and PREUSSE). *Naphthalene* appears to be converted into *oxynaphthalene*, and probably a part also into *dioxynaphthalene* (LESNIK and M. NENCKI). The hydrocarbon with an amino- or imino-group may also be oxidized by a substitution of hydroxyl for hydrogen, especially when the formation of a derivative in the para-position is possible (KLINGENBERG). For example, *aniline*, $C_6H_5.NH_2$, passes into paraminophenol, which latter passes into the urine as its ethereal-sulphuric acid, $H_2N.C_6H_4.O.SO_2.OH$ (F. MÜLLER). *Acetanilid* is in part converted into acetyl paraminophenol (JAFFÉ and HILBERT, K. MÖRNER), and *carbazol* into oxycarbazol (KLINGENBERG).[1]

An *oxidation of the side chain* may occur by the hydrogen atoms being replaced by hydroxyl or may also take place with the formation of carboxyl; thus, for example, *toluene*, $C_6H_5.CH_3$ (SCHULTZEN and NAUNYN), *ethylbenzene*, $C_6H_5.C_2H_5$, and *propylbenzene*, $C_6H_5.C_3H_7$ (NENCKI and GIACOSA),[2] besides many other bodies, are oxidized into benzoic acid. *Cymene* is oxidized to cumic acid, *xylene* to toluic acid, *methylpyridine* to pyridinecarboxylic acid in the same way. If the side chain has several members, the behavior is somewhat different. *Phenylacetic acid*, $C_6H_5.CH_2.COOH$, in which only one carbon atom exists between the benzene nucleus and the carboxyl, is not oxidized, but is eliminated after conjugation with glycocoll as *phenaceturic acid* (SALKOWSKI[3]). *Phenylaminoacetic acid*, $C_6H_5.CHNH_2.COOH$ is in part converted into *mandelic acid* (phenylglycollic acid), $C_6H_5.CHOH.COOH$, and in great part is eliminated as such (SCHOTTEN, KNOOP[4]). *Phenylpropionic acid* $C_6H_5.CH_2.CH_2.COOH$, with three carbon atoms in the side chain, is, on the contrary, oxidized into benzoic acid, and H. and E. SALKOWSKI[5] have proposed the rule that the homologues of the benzoic acids are converted into benzoic acid when the side chain contains more than two carbon atoms.

KNOOP[6] has shown by experiments with several acids, that this rule

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[1] Schultzen and Naunyn, Arch. f. (Anat. u.) Physiol., 1867; Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156. See also Nencki and Giacosa, *ibid.*, **4**; Lesnik and Nencki, Arch. f. exp. Path. u. Pharm., **24**; F. Müller, Deutsch. med. Wochenschr., 1887; Jaffé and Hilbert, Zeitschr. f. physiol. Chem., **12**; Mörner, *ibid.*, **13**; Klingenberg, "Studien über die Oxydation aromatischer Substanzen," etc., Inaug.-Diss. Rostock, 1891. In regard to formanilid, which acts essentially as acetanilid, see Kleine, Zeitschr. f. physiol. Chem., **22**.

[2] Zeitschr. f. physiol. Chem., **4**.

[3] *Ibid.*, **7** and **9**.

[4] *Ibid.*, **8**.

[5] *Ibid.*, **7**.

[6] Hofmeister's Beiträge, **6**, and Habilit.-Schrift, Freiburg, 1904.

does not hold good. He found that the aromatic fatty acids with straight carbon chains such as phenyl butyric acid and phenyl caproic acid, are changed into phenyl acetic acid, which is then conjugated with glycocoll, forming phenylaceturic acid, while the acids with uneven carbon side-chains, such as phenyl propionic and phenylvalerianic acids, yield benzoic acid and are eliminated as hippuric acid. This behavior is explained if, as is made probable by KNOOP, in the demolition those aromatic fatty acids an oxidation takes place in those groups which are in the β-position to the carboxyl group. FRIEDMANN has nevertheless raised objections to this view, but DAKIN,[1] on the other hand, has reported observations which support it strongly. This author found that in the dog phenyl propionic acid was eliminated as hippuric acid, and also a part as phenyl-β-oxypropionic acid, $C_6H_5.CH(OH).CH_2COOH$ and acetophenone, $C_6H_5.CO.CH_3$, which presupposes an oxidation in the β-position. As the last two mentioned bodies can be converted into benzoic acid in the body, DAKIN suggests that the demolition of phenylpropionic acid at least in part occurs with phenyl-β-oxypropionic acid and acetophenone as intemediary steps, and he also undoubtedly finds that in the demolition of the saturated fatty acids that the first stage is the oxidation of the hydrogen combined to a carbon atom in the β-position. KNOOP'S rule does not apply to the propionic acids substituted in the α-position, i.e., phenylalanine, phenyl-α-lactic acid, and phenyl-α-ketopropionic acid, which, like tyrosine and α-amino-cinnamic acid, are burnt in the body. SCHOTTEN'S rule, according to which all acids having three carbon atoms in the side chain of which the middle one has a NH_2 group attached, are almost completely burnt in the organism, has been extended by these exceptions.

If *several side chains* are present in the benzene nucleus, then only one is always oxidized into carboxyl. Thus *xylene*, $C_6H_4(CH_3)_2$, is oxidized into *toluic acid*, $C_6H_4(CH_3)COOH$ (SCHULTZEN and NAUNYN); *mesitylene*, $C_6H_3(CH_3)_3$, into *mesitylenic acid*, $C_6H_3(CH_3)_2.COOH$ (L. NENCKI); *cymene*, $(CH_3)_2CH.C_6H_4.CH_3$, into *cumic acid* (M. NENCKI and ZIEGLER[2]); and *vanillin*, $OH.C_6H_3\begin{cases}OCH_3\\CHO\end{cases}$, into *vanillinic acid* (Y. KOTAKE[3]).

Reductions may also occur and examples of this kind are the conversion, as observed by E. MEYER,[4] of *nitrobenzene*, $C_6H_5NO_2$, or of *nitrophenol*, $HO.C_6H_4.NO_2$ into aminophenol, $HO.C_6H_4.NH_2$, and also the behavior of *m*-nitrobenzaldehyde in the animal body as mentioned below.

[1] Friedmann, Hofmeister's Beiträge, **11**; Dakin, Journ. of biol. Chem., **4**, 419.

[2] L. Nencki, Arch. f. exp. Path. u. Pharm., **1**; Nencki and Ziegler, Ber. d. deutsch. chem., Gesellsch., **5**. See also O. Jacobsen, *ibid.*, **12**.

[3] Zeitschr. f. physiol. Chem., **45**.

[4] *Ibid.*, **46**.

Syntheses of aromatic substances with other atomic groups occur frequently. To these syntheses belongs, in the first place, the conjugation of *benzoic acid* with *glycocoll* to form *hippuric acid*, the discovery of which is generally ascribed to WÖHLER, but according to HEFFTER[1] more correctly to KELLER and URE. All the numerous aromatic substances which are converted into benzoic acid in the body are voided partly as hippuric acid. This statement is not true for all species of animals. According to the observations of JAFFÉ,[2] benzoic acid does not pass into hippuric acid in birds, but into another nitrogenous acid, *ornithuric acid*, $C_{19}H_{20}N_2O_4$. This acid yields as splitting products, besides benzoic acid, *ornithine*, a body which has been spoken of on page 159. Not only are the *oxybenzoic acids* and the *substituted benzoic acids* conjugated with glycocoll, forming corresponding hippuric acids, but also the above-mentioned acids, *toluic*, *mesitylenic*, *cumic*, and *phenylacetic acids*. These acids are voided as *toluric*, *mesitylenuric*, *cuminuric*, and *phenaceturic acids*.

It must be remarked in regard to the oxybenzoic acids that a conjugation with glycocoll has been shown only with salicylic and *p*-oxybenzoic acid (BERTAGNINI, and others), while BAUMANN and HERTER[3] find it only very probable for *m*-oxybenzoic acid. According to BALDONI[4] in dogs the salicylic acid does not pass into salicyluric acid, and he indeed found two acids which he calls ursalicylic acid, $C_{15}N_{14}O_8$ and uraminsalicylic acid, $C_{16}H_{16}NO_8$. The oxybenzoic acids are also in part eliminated as conjugated sulphuric acids, which is especially true for *m*-oxybenzoic acid. The three aminobenzoic acids, according to the experiments of HILDEBRANDT, on rabbits, appeared at least in part unchanged in the urine. SALKOWSKI found, as was later confirmed by R. COHN,[5] that in rabbits *m*-aminobenzoic acid passes in part into *uraminobenzoic acid*, $H_2N.CO.HN.C_6H_4.COOH$. It is also in part eliminated as aminohippuric acid.

The behavior of the halogen-substituted compounds of toluene varies in different animals according to HILDEBRANDT'S experiments. In dogs they are converted into the corresponding substituted hippuric acid. In rabbits *o*-bromtoluene is completely changed to hippuric acid, the *m*- and *p*-bromtoluene only partly. The three chlortoluenes are converted in rabbits into the corresponding benzoic acid and are eliminated as such and not as hippuric acid.

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[1] Die Ausscheidung körperfremder Substanzen im Harn, Ergebnisse der Physiol., **4**, 252.

[2] Ber d. d. chem. Gesellsch., **10** and **11**.

[3] Zeitschr. f. physiol. Chem., **1**, where Bertagnini's work is also cited. See also Dautzenberg, Maly's Jahresber., **11**, 231.

[4] Arch. f. exp. Path. u. Pharm., 1908, Suppl. Bd. (Schmiedeberg's Festschrift).

[5] Salkowski, Zeitschr. f. pyhsiol. Chem., **7**; Cohn, *ibid.*, **17**; Hildebrandt, Hofmeister's Beiträge, **3**.

The substituted aldehydes are of special interest as substances which may undergo conjugation with glycocoll. According to the investigations of R. Cohn [1] on this subject *o-nitrobenzaldehyde* when introduced into a rabbit is only in a very small part converted into nitrobenzoic acid, and the chief mass, about 90 per cent, is destroyed in the body. According to Sieber and Smirnow [2] *m-nitrobenzaldehyde* passes in dogs into *m*-nitrohippuric acid, and according to Cohn into urea-*m*-nitrohippurate, but in rabbits a different action results. In this case not only does an oxidation of the aldehyde into benzoic acid take place, but the nitro-group is also reduced to an amino-group, and finally acetic acid attaches itself to this with the expulsion of water, so that the final product is *m-acetylaminobenzoic acid*, $CH_3.CO.NH.C_6H_4COOH$. This process is analogous to the action of furfurol, and the reduction does not take place in the intestine, but in the tissues. The *p*-nitrobenzaldehyde acts in rabbits in part like the *m*-aldehyde and passes in part into *p-acetylaminobenzoic acid*. Another part is converted into *p*-nitrobenzoic acid, and the urine contains a chemical combination of equal parts of these two acids. According to Sieber and Smirnow *p*-nitrobenzaldehyde yields only urea *p*-nitrohippurate in dogs. The above-mentioned *pyridinecarboxylic acid*, formed from methylpyridine (α-picoline) passes into the urine after conjugation with glycocoll as *α-pyridinuric acid*.[3]

To those substances which undergo a conjugation with glycocoll belongs also *furfurol* (the aldehyde of pyromucic acid), which, when introduced into rabbits and dogs, as shown by Jaffé and Cohn,[4] is first oxidized into pyromucic acid and then, after conjugation with glycocoll, eliminated as *pyromucuric acid*, $C_7H_7NO_4$. In birds this behavior is different, namely, the acid is conjugated with another substance, *ornithine*, $C_5H_{12}N_2O_2$, which is a diaminovaleric acid, forming *pyromucinornithuric acid*.

Furfurol in mammals also undergoes conjugation with glycocoll in other forms. Thus Jaffé and Cohn found that it is in part combined with acetic acid, forming *furfuracrylic acid*, $C_4H_3O.CH:CH.COOH$, which passes into the urine coupled with glycocoll as *furfuracryluric acid*.

It has not been proven how *thiophene*, C_4H_4S, behaves in the animal body. Of *methylthiophene* (thiotolene), $C_4H_3S.CH_3$, a very small part is oxidized to thiophenic acid, $C_4H_3S.COOH$ (Levy). This acid, as shown

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[1] Zeitschr. f. physiol. Chem., **17**.

[2] Monatshefte, f. Chem., 8.

[3] In regard to the extensive literature on glycocoll conjugations we refer the reader to O. Kühling, Ueber Stoffwechselprodukte aromatischer Körper. Inaug.-Diss., Berlin, 1887.

[4] Ber. d. d. Chem. Gesellsch., **20** and **21**.

by JAFFÉ and LEVY,[1] is conjugated with glycocoll in the body (rabbits) and eliminated as *thiophenuric acid*.

Another very important synthesis of aromatic substances is that of the *ethereal-sulphuric acids*. *Phenols* and in particular the *hydroxylated aromatic hydrocarbons* and their derivatives are voided as ethereal-sulphuric acids, according to BAUMANN, HERTER and others.[2]

A conjugation of aromatic acids with sulphuric acid occurs less often. The two previously-mentioned aromatic acids, *p-oxyphenylacetic* and *p-oxyphenylpropionic acid*, are in part eliminated in this form. *Gentisic acid* (hydroquinone-carboxylic acid) also increases, according to LIKHATSCHEFF,[3] the quantity of ethereal-sulphuric acid in the urine, while ROST asserts, contrary to earlier claims, that the same occurs with *gallic acid* (trioxybenzoic acid) and *tannic acid*.[4]

While *acetophenone* (phenylmethylketone), $C_6H_5.CO.CH_3$, as shown by M. NENCKI, is oxidized to benzoic acid and eliminated as hippuric acid, the aromatic oxyketones with hydroxyl groups, such as *resacetophenone*, $C_6H_3(\overset{1}{O}H)(\overset{3}{O}H)(\overset{4}{C}O.CH_3)$, *paraoxypropiophenone*, $C_6H_4(\overset{1}{O}H)(\overset{4}{C}OCH_2.CH_3)$, and *gallacetophenone*, $C_6H_2(\overset{1}{O}H)(\overset{2}{O}H)(\overset{3}{O}H)(\overset{4}{C}O.CH_3)$, pass into the urine without previous oxidation as ethereal-sulphuric acids and in part after conjugation with glucuronic acid (NENCKI and REKOWSKI [5]). *Euxanthon*, which is also an aromatic oxyketone, passes into the urine as *euxanthic acid* after the conjugation with glucuronic acid previously mentioned.

A *conjugation* of other aromatic substances *with glucuronic acid*, which last is protected from combustion, occurs rather often. The phenols, as above stated (page 689), pass in part as conjugated glucuronic acids into the urine. The same is true for the homologues of the phenols, for certain substituted phenols, and for many aromatic substances, also hydrocarbons after previous oxidation and hydration. Thus HILDEBRANDT and FROMM and CLEMENS [6] have shown that the *cyclic terpenes* and *camphors*, by oxidation or hydration, or in certain cases by both, are converted into hydroxyl derivatives when the body in question is not previously hydroxylized, and

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[1] Levy, Ueber das Verhalten einiger Thiophenderivate, etc., Inaug.-Diss., Königsberg, 1889; Jaffé and Levy, Ber. d. d. chem. Gesellsch., **21**.

[2] In regard to the literature, see O. Kühling, l. c.

[3] Zeitschr. f. physiol. Chem., **21**.

[4] In regard to the action of gallic and tannic acids in the animal body, see C. Mörner, Zeitschr. f. physiol. Chem., **16**, which also contains the earlier literature; also Harnack, *ibid.*, **24**, and Rost, Arch. f. exp. Path. u. Pharm., **38**, and Sitzungsber. d. Gesellsch. zur Beförd. d. ges. Naturwiss. zu Marburg, 1898.

[5] Arch. d. scienc. biol. de St. Pétersbourg, **3**, and Ber. d. deutsch. chem. Gesellsch., **27**.

[6] Hildebrandt, Arch. f. exp. Path. u. Pharm., **45**, **46**; Zeitschr. f. physiol. Chem., **36**; with Fromm, *ibid.*, **33**; and with Clemens, *ibid.*, **37**; Fromm and Clemens, *ibid.*, **34**.

that these hydroxyl derivatives are eliminated as conjugated glucuronic acids. Conjugated glucuronic acids are detected in the urine after the introduction of various substances into the organism, e.g., therapeutic agents, such as *terpenes*, *borneol*, *menthol*, *camphor* (camphoglucuronic acid was first observed by SCHMIEDEBERG), *naphthalene*, *oil of turpentine*, *oxyquinolines*, *antipyrine*, and many other bodies.[1] *Orthonitrotoluene* in dogs passes first into *o*-nitrobenzyl alcohol and then into a conjugated glucuronic acid, *uronitrotoluolic acid* (JAFFÉ[2]). The glucuronic acid split off from this conjugated acid is levogyrate and hence is not identical, but only isomeric, with the ordinary glucuronic acid. *Dimethylaminobenzaldehyde*, according to JAFFÉ, is converted in part into *dimethylaminobenzoglucuronic acid* in rabbits. The same conjugated glucuronic acid is also produced, according to HILDEBRANDT,[3] from *p-dimethyltoluidine*, which is first changed into *p-dimethylaminobenzoic acid*. *Indol* and *skatol* seem, as above stated (page 695), to be eliminated in the urine partly as conjugated glucuronic acids.

A synthesis in which compounds containing sulphur, *mercapturic acids*, are formed and eliminated after conjugation with glucuronic acid, occurs when chlorine and bromine derivatives of benzene are introduced into the organism of dogs (BAUMANN and PREUSSE, JAFFÉ). Thus *chlorbenzene* combines with *cysteine*, forming *chlorphenylmercapturic acid*, $C_{11}H_{12}ClSNO_3$. The important investigations of FRIEDMANN[4] show that the phenylthiolactic acid which forms the foundation of the mercapturic acids belongs to the β-series, and in this way the direct chemical connection of this body with the protein-cystine (α-amino-β-thiolactic acid) is established. FRIEDMANN has also been able to convert cysteine into bromphenylmercapturic acid.

Pyridine, C_5H_5N, which does not combine either with glucuronic acid or with sulphuric acid after previous oxidation, shows a special behavior. It takes up a methyl group as found by HIS and later confirmed by COHN, and forms an ammonium combination, *methylpyridylammonium hydroxide*, $HO.CH_3.NC_5H_5$, while in rabbits it occurs unchanged in the urine, according to ABDERHALDEN, BRAHM and SCHITTENHELM.[5]

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[1] See O. Kühling, l. c., which gives the literature up to 1887; also E. Külz, Zeitschr. f. Biologie, **27**; the works of Hildebrandt, Fromm and Clemens, see footnote 6, page 742; Brahm, Zeitschr. f. physiol. Chem., **28**; Fenyvessy, *ibid.*, **30**; Bonanni. Hofmeister's Beiträge, **1**; Lawrow, Ber. d. d. chem. Gesellsch., **33**.

[2] Zeitschr. f. physiol. Chem., **2**.

[3] Jaffé, Zeitschr. f. physiol. Chem., **43**; Hildebrandt, Hofmeister's Beiträge, **7**.

[4] Baumann and Preusse, Zeitschr. f. physiol. Chem., **5**; Jaffé, Ber. d. deutsch. chem. Gesellsch., **12**; Friedmann, Hofmeister's Beiträge, **4**.

[5] His, Arch. f. exp. Path. u. Pharm., **22**; Cohn, Zeitschr. f. physiol. Chem., **18**; Abderhalden and collaborators, *ibid.*, **59**.

Several alkaloids, such as *quinine*, *morphine*, and *strychnine*, may pass into the urine. After the ingestion of *turpentine*, *balsam of copaiba*, and *resins*, these may appear in the urine as resin acids. Different kinds of coloring-matters, such as *alizarin*, *chrysophanic acid*, after rhubarb or senna, and the *coloring-matter of the blueberry*, etc., may also pass into the urine. After *rhubarb*, *senna*, or *santonin* the urine assumes a yellow or greenish-yellow color, which is transformed into a beautiful red by the addition of alkali. *Phenol* produces, as above mentioned, a dark-brown or dark-green color which depends mainly on the decomposition products of hydroquinone and humin substances. After *naphthalene* the urine has a dark color, and several other medicinal agents produce a special coloration. Thus after antipyrine it becomes yellow or blood-red. After *balsam of copaiba* the urine becomes, when strongly acidified with hydrochloric acid, gradually rose- and purple-red. After *naphthalene* or *naphthol* the urine gives with concentrated sulphuric acid (1 cc. of concentrated acid and a few drops of urine) a beautiful emerald-green color, which is probably due to naphthol-glucuronic acid. Odoriferous bodies also pass into the urine. After asparagus the urine acquires a disgusting odor which is probably due to methylmercaptan, according to M. Nencki.[1] After turpentine the urine may have a peculiar odor similar to that of violets.

VI. PATHOLOGICAL CONSTITUENTS OF URINE.

Proteid. The appearance of slight traces of proteid in normal urine has been repeatedly observed by many investigators, such as Posner Plósz, v. Noorden, Leube, and others. According to K. Mörner[2] proteid regularly occurs as a normal urinary constituent to the extent of 22–78 milligrams per liter. Frequently traces of a substance, similar to a nucleoalbumin, which is easily mistaken for mucin, and whose nature will be treated of later, appears in the urine. In diseased conditions proteid occurs in the urine in a variety of cases. The albuminous bodies which most often occur are serglobulin and seralbumin. Proteoses (or peptones) are also sometimes present. The quantity of proteid in the urine is in most cases less than 5 p. m., rarely 10 p. m., and only very rarely does it amount to 50 p. m. or over. Cases are known, however, where it was even more than 80 p. m.

Among the many reactions proposed for the detection of proteid in urine, the following are to be recommended:

The Heat Test. Filter the urine and test its reaction. An acid urine may, as a rule, be boiled without further treatment, and only in especially acid urines is it necessary to first treat with a little alkali. An alkaline urine is made neutral or faintly acid before heating. If the urine is poor in salts, add 1/10 vol. of a saturated common-salt solution before boiling; then heat to the boiling-point, and if no precipitation, cloudiness, or opalescence appears, the urine in question contains no coagulable proteid, but it may contain proteoses or peptones. If a precipitate is produced on boiling, this may consist of proteid, or of earthy phosphates, or of both. The monohydrogen calcium phosphate decomposes on boiling, and the normal phosphate may separate out. The

[1] Arch. f. exp. Path. u. Pharm., 28. [2] Skand. Arch. f. Physiol., 6 (literature).

proper amount of acid is now added to the urine, so as to prevent any mistake caused by the presence of earthy phosphates, and to give a better and more flocculent precipitate of the proteid. If acetic acid is used for this, then add 1–3 drops of a 25 per cent acid to each 10 cc. of the urine and boil after the addition of each drop. On using nitric acid, add 1–2 drops of the 25 per cent acid to each cubic centimeter of the boiling-hot urine.

On using acetic acid, when the quantity of proteid is very small, and especially when the urine was originally alkaline, the proteid may sometimes remain in solution on the addition of the above quantity of acid. If, on the contrary, less acid is added, the precipitate of calcium phosphate, which forms in amphoteric or faintly acid urines, is liable not to dissolve completely, and this may cause it to be mistaken for a proteid precipitate. If nitric acid is used for the heat test, the fact must not be overlooked that after the addition of only a little acid a combination between it and the proteid is formed which is soluble on boiling and which is only precipitated by an excess of the acid. On this account the large quantity of nitric acid, as suggested above, must be added, but in this case a small part of the proteid is liable to be dissolved by the excess of the nitric acid. When the acid is added after boiling, which is absolutely necessary, the liability of a mistake is not so great. It is on these grounds that the heat test, although it gives very good results in the hands of experts, is not recommended to physicians as a positive test for proteid.

A confounding with mucin, when this body occurs in the urine, is easily prevented in the heat test with acetic acid by acidifying another portion with acetic acid at the ordinary temperature. Mucin and nucleoalbumin substances similar to mucin are hereby precipitated. If in the performance of the heat and nitric-acid test a precipitate first appears on cooling or is strikingly increased, then this shows the presence of proteoses in the urine, either alone or mixed with coagulable proteid. In this case a further investigation is necessary (see below). In a urine rich in urates a precipitate consisting of uric acid separates on cooling. This precipitate is colored and granular, and is hardly to be mistaken for a proteose or proteid precipitate.

Heller's *test* is performed as follows (see page 98): The urine is very carefully floated on the surface of nitric acid in a test-tube. The presence of proteid is shown by a white ring between the two liquids. With this test a red or reddish-violet transparent ring is always obtained with normal urine; it depends upon the indigo coloring-matters and can hardly be mistaken for the white or whitish proteid ring, and this last must not be mistaken for the ring produced by bile-pigments. In a urine rich in urates another complication may occur, due to the formation of a ring produced by the precipitation of uric acid. The uric-acid ring does not lie, like the proteid ring, between the two liquids, but somewhat higher. For this reason two simultaneous rings may exist in urines which are rich in urates and do not contain very much proteid. The disturbance caused by uric acid is easily prevented by diluting the urine with 1–2 vols. of water before performing the test. The uric acid now remains in solution, and the delicacy of Heller's test is so great that after dilution only in the presence of insignificant traces of proteid does this test give negative results. In a urine very rich in urea a ring-like separation of urea nitrate may also appear. This ring consists of shining

crystals, and it does not appear in urine previously diluted. A confusion with resinous acids, which also give a whitish ring with this test, is easily prevented, since these acids are soluble on the addition of ether. Stir, add ether, and carefully shake the contents of the test-tube. If the cloudiness is due to resinous acids, the urine gradually becomes clear, and on evaporating the ether a sticky residue of resinous acids is obtained. A liquid which contains true mucin does not give a precipitate with this test, but it gives a more or less strongly opalescent ring, which disappears on stirring. The liquid does not contain any precipitate after stirring, but is somewhat opalescent. If a faint, not wholly typical reaction is, obtained with HELLER'S test after some time with undiluted urine, while the diluted urine gives a pronounced reaction, the presence is shown of the substance which used to be called mucin or nucleoalbumin. In this case proceed as described below for the detection of nucleoalbumin.

If the above-mentioned possible errors and the means by which they may be prevented are borne in mind, there is hardly another test for proteid in the urine which is at the same time so easily performed, so delicate, and so positive as HELLER'S. With this test even 0.002 per cent of albumin may be detected without difficulty. Still the student must not be satisfied with this test alone, but should apply at least a second one, such as the heat test. In performing this test the (primary) proteoses are also precipitated.

The *reaction with metaphosphoric acid* (see page 98) is very convenient and easily performed. It is not quite so delicate and positive as HELLER'S test. The proteoses are also precipitated by this reagent.

Reaction with Acetic Acid and Potassium Ferrocyanide. Treat the urine first with acetic acid until it contains about 2 per cent, and then add drop by drop a potassium-ferrocyanide solution (1:20), carefully avoiding an excess. This test is very good, and in the hands of experts it is even more delicate than HELLER'S. In the presence of a very small quantities of proteid it requires more practice and dexterity than HELLER'S as the relative quantities of reagent, proteid, and acetic acid influence the result of the test. The quantity of salts in the urine likewise seems to have an influence. This reagent also precipitates proteoses.

SPIEGLER'S *Test.* SPIEGLER recommends a solution of 8 parts mercuric chloride, 4 parts tartaric acid, 20 parts glycerin, and 200 parts water as a very delicate reagent for proteid in the urine. A test-tube is half filled with this reagent and the urine is allowed to flow upon its surface drop by drop from a pipette along the wall of the test-tube. In the presence of proteid a white ring is obtained at the point of contact between the two liquids. The delicacy of this test is 1:350,000. JOLLES [1] does not consider this reagent suited for urines very poor in chlorine, and for this reason he has changed it as follows: 10 grams mercuric chloride, 20 grams succinic acid, 10 grams NaCl, and 500 cc. water.

ROCH'S *Test.* Treat the urine either with a 20 per cent watery solution of sulphosalicylic acid or a few crystals of the acid. This reagent does not precipitate the uric acid or the resin acids.[2]

As every normal urine contains traces of proteid, it is apparent that very delicate reagents are to be used only with the greatest caution. For

[1] Spiegler, Wien. klin. Wochenschr., 1892, and Centralbl. f. d. klin. Med., 1893; Jolles, Zeitschr. f. physiol. Chem., **21**.

[2] Pharmaceut. Centralbl., 1889, and Zeitschr. f. physiol. Chem., **29**.

ordinary cases HELLER'S test is sufficiently delicate. If no reaction is obtained with this test within 2½ to 3 minutes, the urine tested contains less than 0.003 per cent of proteid, and is to be considered free from proteid in the ordinary sense.

The use of precipitating reagents presumes that the urine to be investigated is perfectly clear, especially in the presence of only very little proteid. The urine must first be filtered. This is not easily done with urine containing bacteria, but a clear urine may be obtained, as suggested by A. JOLLES, by shaking the urine with infusorial earth. Although a little proteid is retained in this procedure and lost, it does not seem to be of any importance (GRÜTZNER, SCHWEISSINGER [1]).

The different *color reactions* cannot be directly used, especially in deep-colored urines which contain only little proteid. The common salt of the urine has a disturbing action on MILLON'S reagent. To prove more positively the presence of proteid, the precipitate obtained in the boiling test may be filtered, washed, and then tested with MILLON'S reagent. The precipitate may also be dissolved in dilute alkali and the biuret test applied to the solution. The presence of proteoses or peptones in the urine is directly tested for by this last-mentioned test.

In testing the urine for proteid one should never be satisfied with one reaction alone, but must apply the heat test and HELLER'S or the potassium-ferrocyanide test. In using the heat test alone the proteoses may be easily overlooked, but these are detected, on the contrary, by HELLER'S or the potassium-ferrocyanide test. If only one of these tests is employed, no sufficient intimation of the kind of proteid present can be obtained, whether it consists of proteoses or coagulable proteid.

For practical purposes several dry reagents for proteid have been recommended. Besides the metaphosphoric acid may be mentioned STUTZ'S or FÜRBRINGER'S gelatin capsules, which contain mercuric chloride, sodium chloride, and citric acid; and GEISSLER'S albumin-test papers, which consist of strips of filter-paper some of which have been dipped in a solution of citric acid, and some into a solution of mercuric-chloride and potassium-iodide solution, and then dried.

If the presence of proteid has been positively proven in the urine by the above tests, it then remains necessary to determine its character.

The *Detection of Globulin and Albumin.* In detecting serglobulin the urine is exactly neutralized, filtered, and treated with magnesium sulphate in substance until it is completely saturated at the ordinary temperature, or with an equal volume of a saturated neutral solution of ammonium sulphate. In both cases a white, flocculent precipitate is formed in the presence of globulin. In using ammonium sulphate with a urine rich in urates a precipitate consisting of ammonium urate may separate. This precipitate does not appear immediately, but only after a certain time, and it must not be mistaken for the globulin precipitate. In detecting seralbumin heat the filtrate from the globulin precipitate to boiling-point, or add about 1 per cent acetic acid to it at the ordinary temperature.

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[1] Jolles, Zeitschr. f. anal. Chem., **29**; Grützner, Chem. Centralbl., 1901, **1**; Schweissinger, *ibid.*

For the detection and also for the quantitative estimation of the various globulins (fibringlobulin, euglobulin, and pseudoglobulin) Oswald [1] has proposed the fractional precipitation with ammonium sulphate.

Proteoses and *peptones* have been repeatedly found in the urine in different diseases. Reliable reports are at hand on the occurrence of proteoses in the urine. The statements in regard to the occurrence of peptones date from a time when the conception of proteoses and peptones was different from that of the present day, and in part they are based upon investigations using untrustworthy methods. According to Ito [2] true peptones are sometimes found in the urine in cases of pneumonia; what has been designated as urine peptones seems to have been chiefly deuteroproteoses.

In detecting the proteoses the proteid-free urine, or urine boiled with addition of acetic acid, is saturated with ammonium sulphate, which precipitates the proteoses. Several errors are here possible. The urobilin, which may give a reaction similar to the biuret reaction, is also precipitated and may lead to mistakes (Salkowski, Stokvis [3]). The following modification by Bang of Devoto's [4] method can be used to advantage: The urine is heated to boiling with ammonium sulphate (8 parts to 10 parts urine) and boiled for a few seconds. The hot liquid is centrifuged for ½ to 1 minute and separated from the sediment. The urobilin is removed from this by extraction with alcohol. The residue is suspended in a little water, heated to boiling, filtered, whereby the coagulable proteid is retained on the filter, and any urobilin still present in the filtrate is shaken out with chloroform. The watery solution, after removal of the chloroform, is used for the biuret test. For clinical purposes this method is very serviceable.

According to Salkowski the urine treated with 10-per cent hydrochloric acid is precipitated with phosphotungstic acid, then warmed, the liquid decanted from the resin-like precipitate, this washed with water, and then dissolved in a little water with the aid of some caustic soda, warmed again until the blue color disappears, cooled, and finally tested with copper sulphate. This method has been recently somewhat modified by v. Aldor and Cerny.[5] In regard to other more complicated methods we refer to Huppert-Neubauer.

Morawitz and Dietschy [6] first remove the proteid from the urine made faintly acid with acid potassium phosphate by the addition of double the volume of 96-per cent alcohol and warming on the water-bath for several hours. From the concentrated filtrate acidified with a little sulphuric acid the proteoses can be precipitated by saturating with zinc sulphate. After the removal of the urobilin by alcohol and extracting with water, the biuret test may be applied.

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[1] Münch. med. Wochenschr., 1904. See also Zak and Necker, Deutsch. Arch. f. klin. Med., 88.

[2] In regard to the literature on proteoses and peptones in urine, see Huppert-Neubauer, Harn-Analyse, 10. Aufl., 466 to 492; also A. Stoffregen, Ueber das Vorkommen von Pepton im Harn, Sputum und Eiter (Inaug.-Diss., Dorpat, 1891); E. Hirschfeldt, Ein Beitrag zur Frage der Peptonurie (Inaug.-Diss., Dorpat, 1892); and especially Stadelmann, Untersuchungen über die Peptonurie. Wiesbaden, 1894; Ehrström, Bidrag till kännedomen om Albumosurien, Helsingfors, 1900; Ito, Deutsch. Arch. f. klin. Med., **71**.

[3] Salkowski, Berlin. klin. Wochenschr., 1897; Stokvis, Zeitschr. f. Biologie, **34**.

[4] Devoto, Zeitschr. f. physiol. Chem., **15**; Bang, Detusch. med. Wochenschr., 1898

[5] Salkowski, Centralbl. f. d. med. Wissensch., 1894; v. Aldor, Berl. klin. Wochenschr., **36**; Černy, Zeitschr. f. analyt. Chem., **40**.

[6] Arch. f. exp. Path. u. Pharm., **54**.

If the proteoses have been precipitated from a larger portion of urine by ammonium sulphate, this precipitate is tested for the presence of different proteoses for the reasons given in Chapter III. The following serves as a preliminary determination of the character of the proteoses present in the urine. If the urine contains only deuteroproteose it does not become cloudy on boiling, does not give HELLER'S test, does not become cloudy on saturating with NaCl in neutral reaction, but does become turbid on adding acetic acid saturated with this salt. In the presence of only protoproteose the urine gives HELLER'S test, is precipitated even in neutral solution on saturating with NaCl, but does not coagulate on boiling. The presence of heteroproteose is shown by the urine behaving like the above with NaCl and nitric acid, but shows a difference on heating. It gradually becomes cloudy on warming and separates at about 60° C. a sticky precipitate which attaches itself to the sides of the vessel and which dissolves at boiling temperature on acidifying the urine; the precipitate reappears on cooling.

In close relation to the proteoses stands the so-called BENCE-JONES proteid, which occurs in the urine in rare cases in diseases with changes in the spinal marrow. It gives a precipitate on heating to 40–60° C., which on further heating to boiling dissolves again more or less completely, depending upon the reaction and upon the amount of salt present. It does not separate on dialysis, but can be precipitated from the urine by double the volume of a saturated ammonium-sulphate solution or by alcohol. It has also been obtained as crystals (GRUTTERINK and DE GRAAFF, MAGNUS-LEVY). This body shows a varying behavior in the different cases in which it has been found and its nature has not been explained. From the investigations of the above-mentioned and other experimenters (MOITESSIER, ABDERHALDEN and ROSTOSKI) we can draw the conclusion that this proteid is similar to the proteoses in several reactions, but that nevertheless it stands close to the genuine protein bodies. It also yields primary as well as secondary proteoses on peptic digestion (GRUTTERINK and DE GRAAFF), and yields the same hydrolytic cleavage products as the other proteins (ABDERHALDEN and ROSTOSKI).[1]

Quantitative Estimation of Proteid in Urine. Of all the methods proposed thus far, the COAGULATION METHOD (boiling with the addition of acetic acid) when performed with sufficient care gives the best results. The average error need never amount to more than 0.01 per cent, and it is generally smaller. With this method it is best to first find how much acetic acid must be added to a small portion of the urine, which has been previously heated on the water-bath, to completely separate the proteid so that the filtrate will not respond to HELLER'S test. Then coagulate 20–50–100 cc. of the urine. Pour the urine into a beaker and heat on the water-bath, add the required quantity of acetic acid slowly, stirring constantly, and heat at the same time. Filter while warm, wash first with water, then with alcohol and ether, dry and weigh, incinerate and weigh again. In exact determinations the filtrate must not give HELLER'S test.

The separate estimation of GLOBULINS and ALBUMINS is done by carefully neutralizing the urine and precipitating with $MgSO_4$ added to saturation (HAMMAR-

[1] Magnus-Levy, Zeitschr. f. physiol. Chem., **30** (literature); Grutterink and de Graaff, *ibid.*, **34** and **46**; Moitessier, Compt. rend. soc. biolog., **57**; Ville and Derrien, *ibid.*, **62**; Abderhalden and Rostoski, Zeitschr. f. physiol. Chem., **46**.

STEN), or simply by adding an equal volume of a saturated neutral solution of ammonium sulphate (HOFMEISTER and POHL [1]). The precipitate consisting of globulin is thoroughly washed with a saturated magnesium-sulphate or half-saturated ammonium-sulphate solution, dried continuously at 110° C., boiled with water, extracted with alcohol and ether, then dried, weighed, incinerated, and weighed again. The quantity of albumin is calculated as the difference between the quantity of globulin and the total proteids.

Approximate Estimation of Proteid in Urine. Of the methods suggested for this purpose none has been more extensively employed than ESBACH'S.

ESBACH'S [2] *Method.* The acidified urine (with acetic acid) is poured into a specially graduated tube to a certain mark, and then the reagent (a 2-per cent citric-acid and 1-per cent picric-acid solution in water) is added to a second mark, the tube closed with a rubber stopper and carefully shaken, avoiding the production of froth. The tube is allowed to stand twenty-four hours, and then the height of the precipitate on the graduation is read off. The reading gives directly the quantity of proteid in 1000 parts of the urine. Urines rich in proteid must first be diluted with water. The results obtained by this method are, however, dependent upon the temperature; and a difference in temperature of 5° to 6.5° C. may cause an error of 0.2–0.3 per cent deficiency or excess in urines containing a medium quantity of proteid (CHRISTENSEN and MYGGE). The method suggested by TSUSCHIJA[3] seems to be more reliable and consists in precipitating the proteid by an alcoholic solution of phosphotungstic acid containing hydrochloric acid.

Other methods for the approximate estimation of proteid are the optical methods of CHRISTENSEN and MYGGE, and of WALBUM,[4] of ROBERTS and STOLNIKOW as modified by BRANDBERG, with HELLER'S test, which has been simplified for practical purposes by MITTELBACH. The density methods of LANG, HUPPERT, and ZAHOR are also very good. In regard to these and other methods we refer to HUPPERT-NEUBAUER'S Harn-Analyse, 10. Aufl.

There is at present no trustworthy method for the quantitative estimation of proteoses and peptone in the urine.

Nucleoalbumin and Mucin. According to K. MÖRNER traces of urinary mucoids may pass into solution in the urine; otherwise normal urine contains no mucin. There is no doubt that there may be cases where true mucin appears in the urine; in most cases mucin has probably been mistaken for so-called nucleoalbumin. The occurrence, under some circumstances, of nucleoalbumin in the urine is not to be denied, as such substances occur in the renal and urinary passages; still in most cases this nucleoalbumin, as shown by K. MÖRNER,[5] is of an entirely different kind.

All urine, according to MÖRNER, contains a little proteid and in addition substances precipitating proteid. If the urine freed from salts by dialysis is shaken with choloroform after the addition of 1–2 p. m. acetic acid, a precipitate is obtained which acts like a nucleoalbumin.

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[1] Hammarsten, Pflüger's Arch., **17**; Hofmeister and Pohl, Arch. f. exp. Path. u. Pharm., **20**.

[2] In regard to the literature on this method and the numerous experiments to determine its value, see Huppert-Neubauer, 10 Aufl., 853.

[3] Christensen, Virchow's Arch., **115**; Tsuschija, Centralbl. f. Med., 1908.

[4] Detusch. med. Wochenschr., 1908.

[5] Skand. Arch. f. Physiol., **6**.

If the acid filtrate is treated with seralbumin, a new and similar precipitate is obtained, due to the presence of a residue of the substance which precipitates proteids. The most important of these proteid-precipitating substances is chrondroitin-sulphuric acid and nucleic acid, although the latter appears to a much smaller extent. Taurocholic acid may in a few instances, especially in icteric urines, be precipitated. The substances isolated by different investigators from urine by the addition of acetic acid and called " dissolved mucin " or " nucleoalbumin " are considered by MÖRNER to be a combination of proteid chiefly with chrondroitin-sulphuric acid, and to a less extent with nucleic acid, and also perhaps with taurocholic acid.

As normal urine habitually contains an excess of substances capable of precipitating proteids, it is apparent that an increased elimination of so-called nucleoalbumin may be caused simply by an augmented excretion of proteid. This happens to a still greater extent in cases where the proteid as well as the proteid-precipitating substance is eliminated to an increased extent.

Detection of so-called Nucleoalbumins. When a urine becomes cloudy or precipitates on the addition of acetic acid, and when it gives a more typical reaction with HELLER'S test after the dilution of the urine than before, one is justified in making tests for mucin and nucleoalbumin. As the salts of the urine interfere considerably with the precipitation of these substances by acetic acid, they must first be removed by dialysis. As large a quantity of urine as possible is dialyzed (with the addition of chloroform) until the salts are removed. The acetic acid is added until it contains 2 p. m., and the mixture allowed to stand. The precipitate is dissolved in water by the aid of the smallest possible quantity of alkali and precipitated again. In testing for chrondroitin-sulphuric acid a part is warmed on the water-bath with about 5 per cent hydrochloric acid. If positive results are obtained on testing for sulphuric acid and reducing substance, then chondroproteid was present. If a reducing substance can be detected but no sulphuric acid, then mucin is probably there. If it does not contain any sulphuric acid or reducing substance, a part of the precipitate is exposed to pepsin digestion and another part used for the determination of any organic phosphorus. If positive results are obtained from these tests, then nucleoalbumin and nucleoproteid must be differentiated by special tests for nuclein bases. No positive conclusion can be drawn except by using very large quantities of urine. The filtrate from the nucleoalbumin can be used for the ordinary proteid tests.

Nucleohistone. In a case of pseudoleucæmia A. JOLLES found a phosphorized protein substance which he considers as identical with nucleohistone. *Histone* is claimed to have been found in some cases by KREHL and MATTHES, and by KOLISCH and BURIAN.[1]

[1] Jolles, Ber. d. deutsch. chem. Gesellsch., **30**; Krehl and Matthes, Deutsch. Arch. f. klin. Med., **54**; Kolisch and Burian, Zeitschr. f. klin. Med., **29**.

Blood and Blood-coloring Matters. The urine may contain blood from hemorrhage in the kidneys or other parts of the urinary passages (HÆMATURIA). In these cases, when the quantity of blood is not very small, the urine is more or less cloudy and colored reddish, yellowish red, dirty red, brownish red, or dark brown. In recent hemorrhages in which the blood has not decomposed the color is nearer blood-red. Blood-corpuscles may be found in the sediment, sometimes also blood-casts and smaller or larger blood-clots.

In certain cases the urine contains no blood-corpuscles, but only dissolved blood-coloring matters, hæmoglobin, or, and indeed quite often, methæmoglobin (HÆMOGLOBINURIA). The blood-pigments appear in the urine under different conditions, as in dissolution of blood in poisoning with arseniuretted hydrogen, chlorates, etc., after serious burns, after transfusion of blood, and also in the periodic appearance of hæmoglobinuria with fever. In hæmoglobinuria the urine may also have an abundant grayish-brown sediment rich in proteid which contains the remains of the stromata of the red blood-corpuscles. In animals, hæmoglobinuria may be produced by many causes which force free hæmoglobin into the plasma.

To detect blood in the urine, we make use of the microscope, the spectroscope, the guaiac test, and HELLER'S or HELLER-TEICHMANN'S test.

Microscopic Investigation. The blood-corpuscles may remain undissolved for a long time in acid urine; in alkaline urine, on the contrary, they are easily changed and dissolved. They often appear entirely unchanged in the sediment; in some cases they are distended and in others unequally pointed or jagged like a thorn-apple. In hemorrhage of the kidneys a cylindrical clot is sometimes found in the sediment which is covered with numerous red blood-corpuscles, forming casts of the urinary passages. These formations are called *blood-casts.*

The *spectroscopic investigation* is naturally of very great value; and if it be necessary to determine not only the presence but also the kind of coloring-matter, this method is indispensable. In regard to the optical behavior of the various blood-pigments we must refer to Chapter VI.

Guaiac Test. Mix in a test-tube equal volumes of tincture of guaiac and old turpentine which has become strongly ozonized by the action of air under the influence of light. To this mixture, which must not have the slightest blue color, add the urine to be tested. In the presence of blood or blood-pigments, first a bluish-green and then a beautiful blue ring appears where the two liquids meet. On shaking the mixture it becomes more or less blue. Normal urine or one containing proteid does not give this reaction. According to LIEBERMANN [1] this reaction is brought about by the blood pigments acting as catalyst upon the organic peroxides existing in the turpentine, accelerating the decomposi-

[1] Pflüger's Arch., **104.**

tion of these and the active oxygen taken up by the guaiaconic acid which is oxidized to guaiac blue (guaiaconic acid ozonide). Urine containing pus, even when no blood is present, gives a blue color with these reagents; but in this case the tincture of guaiac alone, without turpentine, is colored blue by the urine (VITALI [1]). This is at least true for a tincture that has been exposed for some time to the action of air and sunlight. The blue color produced by pus differs from that produced by blood-coloring matters by disappearing on heating the urine to boiling. A urine alkaline by decomposition must first be made faintly acid before performing the reaction. The turpentine should be kept exposed to sunlight, while the tincture of guaiac must be kept in a dark glass bottle. These reagents to be of use must be controlled by a liquid containing blood. With positive results, however, this test is not absolutely decisive, because other bodies may give a similar reaction, but when properly performed it is so extremely delicate that when it gives negative results any other test for blood is superfluous.[2]

HELLER-TEICHMANN'S *Test.* If a neutral or faintly acid urine containing blood is heated to boiling, one always obtains a mottled precipitate consisting of proteid and hæmatin. If caustic soda is added to the boiling hot test, the liquid becomes clear and turns green when examined in thin layers (due to hæmatin alkali), and a red precipitate, appearing green by reflected light, re-forms, consisting of earthy phosphates and hæmatin. This reaction is called HELLER'S blood-test. If this precipitate is after a time collected on a small filter, it may be used for the hæmin test (see page 286). If the precipitate contains only a little blood-coloring matter with a larger quantity of earthy phosphates, then wash it with dilute acetic acid, which dissolves the earthy phosphates, and use the residue for the preparation of TEICHMANN'S hæmin crystals. If, on the contrary, the amount of phosphates is very small, then first add a little $MgCl_2$ solution to the urine, heat to boiling, and add simultaneously with the caustic potash some sodium-phosphate solution. In the presence of only very small quantities of blood, first make the urine very faintly alkaline with ammonia, add tannic acid, acidify with acetic acid, and use this precipitate in the preparation of the hæmin crystals (STRUVE [3]).

O. and R. ADLER [4] have recommended leucomalachite green or benzidine in the presence of peroxide and acetic acid as especially sensitive reagents for blood.

Hæmatoporphyrin. Since the occurrence of hæmatoporphyrin in the urine in various diseases has been made very probable by several investigators, such as NEUSSER, STOKVIS, MACMUNN, LE NOBEL, COPEMAN, and others,[5] SALKOWSKI has positively shown the presence of this pigment

[1] See Maly's Jahresber., 18.

[2] For more details in regard to the preparation of the reagents and the performance of the reaction see O. Schumm, Zeitschr. f. physiol. Chem., 50.

[3] Zeitschr. f. anal. Chem., 11.

[4] Zeitschr. f. physiol. Chem., 41.

[5] A very complete index of the literature on hæmatoporphyrin in the urine may be found in R. Zoja, Su qualche pigmento di alcune urine, etc., in Arch. Ital. di. clin. Med., 1893.

in the urine after sulfonal intoxication. It was first isolated in a pure crystalline state by HAMMARSTEN [1] from the urine of insane women after sulfonal intoxication. According to GARROD and SAILLET [2] traces of hæmatoporphyrin (SAILLET'S urospectrin) regularly occur in normal urines. It is also found in the urine during different diseases, although it occurs only in small quantities. It has been found in considerable quantities in the urine after the lengthy use of sulfonal.

Urine containing hæmatoporphyrin is sometimes only slightly colored, while in other cases, as for example, after the use of sulfonal, it is more or less deep red. In these last-mentioned cases the color depends, in greatest part, not upon the hæmatoporphyrin, but upon other red or reddish-brown pigments which have not been sufficiently studied.

In the detection of small quantities of hæmatoporphyrin proceed as suggested by GARROD. Precipitate the urine with a 10-per cent caustic-soda solution (20 cc. for every 100 cc. of urine). The phosphate precipitate containing the pigment is dissolved in alcohol-hydrochloric acid (15–20 cc.) and the solution investigated by the spectroscope. In more exact investigations make the solution alkaline with ammonia, add enough acetic acid to dissolve the phosphate precipitate, shake with chloroform, which takes up the pigment, and test this solution with the spectroscope.

In the presence of larger quantities of hæmatoporphyrin the urine is first precipitated, according to SALKOWSKI, with an alkaline barium-chloride solution (a mixture of equal volumes of barium-hydroxide solution, saturated in the cold, and a 10-per cent barium-chloride solution), or, according to HAMMARSTEN,[3] with a barium-acetate solution. The washed precipitate, which contains the hæmatoporphyrin, is allowed to stand some time at the temperature of the room, with alcohol containing hydrochloric or sulphuric acid, and then filtered. The filtrate shows the characteristic spectrum of hæmatoporphyrin in acid solution and gives the spectrum of alkaline hæmatoporphyrin after saturation with ammonia. If the alcoholic solution is mixed with chloroform and a large quantity of water added and carefully shaken, sometimes a lower layer of chloroform is obtained which contains very pure hæmatoporphyrin, while the upper layer of alcohol and water contains the other pigments besides some hæmatoporphyrin.

Other methods which have no advantage over this one of GARROD have been suggested by RIVA and ZOJA as well as SAILLET.[4]

BAUMSTARK [5] found in a case of leprosy two characteristic coloring-matters in the urine, "urorubrohæmatin" and "urofuscohæmatin," which, as their names indicate, seem to stand in close relation to the blood-coloring matters. *Urorubrohæmatin*, $C_{68}H_{94}N_8Fe_2O_{26}$, contains iron and shows in acid solution an absorp-

[1] Salkowski, Zeitschr. f. physiol. Chem., **15**; Hammarsten, Skand. Arch. f. Physiol., **3**

[2] Garrod, Journ. of Physiol., **13** (contains review of literature) and **17**; Saillet, Revue de Médecine, **16**.

[3] Salkowski, l. c.; Hammarsten, l. c.

[4] Riva and Zoja, Maly's Jahresber., **24**; Saillet, l. c. See also Nebelthau, Zeitschr. f. physiol. Chem., **27**.

[5] Pflüger's Arch., **9**.

tion-band in front of *D* and a broader one back of *D*. In alkaline solution it shows four bands—behind *D*, at *E*, beyond *F*, and behind *G*. It is not soluble either in water, alcohol, ether, or chloroform. It gives a beautiful brownish-red non-dichroic liquid with alkalies. *Urofuscohæmatin*, $C_{68}H_{106}N_8O_{26}$, which is free from iron, shows no characteristic spectrum; it dissolves in alkalies, producing a brown color. It remains to be proven whether these two pigments are related to (impure) hæmatoporphyrin.

Melanin. In the presence of melanotic cancers dark pigments are sometimes eliminated with the urine. K. MÖRNER has isolated two pigments from such a urine, of which one was soluble in warm 50–75 per cent acetic acid, while the other, on the contrary, was insoluble. The one seemed to be *phymatorhusin* (see Chapter XVI). Usually the urine does not contain any melanin, but a chromogen of melanin, a *melanogen*. In such cases the urine gives EISLET'S reaction, becoming dark-colored with oxidizing agents, such as concentrated nitric acid, potassium bichromate, and sulphuric acid, as well as with free sulphuric acid. Urine containing melanin or melanogen is colored black by a ferric-chloride solution (v. JAKSCH [1]).

Pus occurs in the urine in various inflammatory affections, especially in catarrh of the bladder and in inflammation of the pelvis of the kidneys or of the urethra.

Pus is best detected by means of the miscroscope. The pus-cells are rather easily destroyed in alkaline urines. In detecting pus we make use of DONNÉ'S pus test, which is performed in the following way: Pour off the urine from the sediment as carefully as possible, place a small piece of caustic alkali on the sediment, and stir. If the pus-cells have not been previously changed, the sediment is converted by this means into a slimy tough mass.

The pus-corpuscles swell up in alkaline urines, and dissolve, or at least are so changed that they cannot be recognized under the microscope. The urine in these cases is more or less slimy or fibrous, and the proteid can be precipitated in large flakes by acetic acid, so that it might possibly be mistaken for mucin. The closer investigation of the precipitate produced by acetic acid, and especially the appearance or non-appearance of a reducing substance after boiling it with a mineral acid, demonstrates the nature of the precipitated substance. Urine containing pus always contains proteid.

Bile-acids. The reports in regard to the occurrence of bile-acids in the urine under physiological conditions do not agree. According to DRAGENDORFF and HÖNE traces of bile-acids occur in the urine; according to MACKAY and v. UDRÁNSZKY and K. MÖRNER [2] they do not. Pathologically they are present in the urine in hepatogenic icterus, although not invariably.

Detection of Bile-acids in the Urine. PETTENKOFER'S test gives the most decisive reaction; but as it gives similar color reactions with other bodies, it must be supplemented by the spectroscopic investigation. The direct test for bile-acids is easily performed after the addition of traces of bile to a normal urine.

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[1] K. Mörner, Zeitschr. f. physiol. Chem., **11**; v. Jaksch, *ibid.*, **13**.

[2] Cited from Huppert-Neubauer, Harn-Analyse, 10. Aufl. 229.

But the direct detection in a colored icteric urine is more difficult and gives very misleading results; the bile-acid must therefore always be isolated from the urine. This may be done by the following method of HOPPE-SEYLER, which is slightly modified in non-essential points.

HOPPE-SEYLER'S METHOD. Concentrate the urine and extract the residue with strong alcohol. The filtrate is freed from alcohol by evaporation and then precipitated by basic lead acetate and ammonia. The washed precipitate is treated with boiling alcohol, filtered hot, the filtrate treated with a few drops of soda solution, and evaporated to dryness. The dry residue is extracted with absolute alcohol, filtered, and an excess of ether added. The amorphous or, after a longer time, crystalline, precipitate consisting of the alkali salts of the biliary acids is used in performing PETTENKOFER'S test.

HAYCRAFT has suggested a reaction for clinical purposes which consists in sprinkling flowers of sulphur upon the urine. In icteric urine the powder quickly sinks to the bottom, while in normal urine is remains on the surface. The value of this test is still questioned.

Bile-pigments occur in the urine in different forms of icterus. A urine containing bile-pigments is always abnormally colored—yellow, yellowish brown, deep brown, greenish yellow, greenish brown, or nearly pure green. On shaking it froths and the bubbles are yellow or yellowish green in color. As a rule icteric urine is somewhat cloudy, and the sediment is frequently, especially when it contains epithelium-cells, rather strongly colored by the bile-pigments. In regard to the occurrence of urobilin in icteric urine see p. 707.

Detection of Bile-coloring Matters in Urine. Many tests have been proposed for the detection of these substances. Ordinarily we obtain the best results either with GMELIN'S or with HUPPERT'S test.

GMELIN'S *test* may be applied directly to the urine; but it is better to use ROSENBACH'S modification. Filter the urine through a very small filter, which becomes deeply colored from the retained epithelium-cells and bodies of that nature. After the liquid has entirely passed through apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale-yellow spot will be formed which is surrounded by colored rings which appear yellowish red, violet, blue, and green from within outward. This modification is very delicate, and it is hardly possible to mistake indican and other coloring-matters for the bile-pigments. Several other modifications of GMELIN'S direct test, e.g., with concentrated sulphuric acid and nitrate, etc., have been proposed, but they are neither simpler nor more delicate than ROSENBACH'S modification.

HUPPERT'S *Reaction.* In a dark-colored urine or one rich in indican good results are not always obtained with GMELIN'S test. In such cases, as also in urines containing blood-coloring matters at the same time, the urine is treated with lime-water, or first with some $CaCl_2$ solution, and then with a solution of sodium or ammonium carbonate. The precipitate which contains the bile-coloring matter is filtered, washed, dissolved in alcohol which contains 5 cc. of concentrated hydrochloric acid in 100

cc. (I. Munk), and heated to boiling when the solution becomes green or bluish green. According to Nakayama [1] this reaction is more delicate on using a mixture of ferric chloride, acid, and alcohol.

Hammarsten's *Reaction.* For ordinary cases it is sufficient to add a few drops of urine to about 2–3 cc. of the reagent (see page 408), when the mixture immediately after shaking turns a beautiful green or bluish green, which color remains for several days. In the presence of only very small quantities of bile-pigments, especially when blood or other pigments are simultaneously present, pour about 10 cc. of the acid or nearly neutral (not alkaline) urine into the tube of a small centrifugal machine and add $BaCl_2$ solution and centrifuge for about one minute. The liquid is decanted and the sediment stirred with about 1 cc. of the reagent and centrifuged again. A beautiful green solution is obtained, which may be changed by the addition of increased quantities of the acid mixture to blue, violet, red, and reddish yellow. The green color may be obtained in the presence of 1 part bile-pigment in 500,000–1,000,000 parts urine. In the presence of large amounts of other pigments calcium chloride is better suited than barium chloride.

Bouma [2] has suggested the use of alcohol containing ferric chloride and hydrochloric acid instead of the above-mentioned acid mixture. He has also worked out a colorimetric method of quantitative estimation of bilirubin in urine by means of this reagent.

As above indicated, we have a great many other tests besides these given above. A very complete summary of these tests and the literature thereof can be found in the work of Obermayer and Popper.[3]

For ordinary purposes the above-mentioned tests are sufficiently delicate, and according to Hammarsten it is not advisable, as also in the case of the detection of proteid, sugar, etc., to increase the delicacy of a test so that it shows the presence of the traces of the questionable substance in normal urine. If in certain cases a greater delicacy is required than is obtained with the above tests, then we must recommend the flotation test of Obermayer and Popper with iodine and salt.

Medicinal coloring-matters produced from santonin, rhubarb, senna, etc., may give an abnormal color to the urine and may be mistaken for bile-pigments, or, in alkaline urines, perhaps for blood-coloring matters. If hydrochloric acid is added to such a urine, it becomes yellow or pale yellow, while on the addition of an excess of alkali it takes on a more or less beautiful red color.

SUGAR IN URINE.

The occurrence of traces of dextrose in the urine of perfectly healthy persons has been, as above stated (page 711), quite positively proven. If sugar appears in the urine in constant and especially in large quantities, it must be considered as an abnormal constituent. In a previous chapter several of the principal causes of glycosuria in man and animals were men-

[1] Munk, Arch. f. (Anat. u.) Physiol., 1898; Nakayama, Zeitschr. f. physiol. Chem., 36.

[2] Deutsch. med. Wochenschr., 1902 and 1904.

[3] Wien. med. Wochenschr., 1902 and 1904.

tioned, and the reader is referred to Chapters VIII and IX for the essential facts in regard to the appearance of sugar in the urine.

In man the appearance of dextrose in the urine has been observed under various pathological conditions, such as lesions of the brain and especially of the medulla oblongata, abnormal circulation in the abdomen, diseases of the heart, lungs and liver, cholera, and many other diseases. The continued presence of sugar in human urine, sometimes in very considerable quantities, occurs in DIABETES MELLITUS. In this disease there may be an elimination of 1 kilogram or even more of dextrose per day. In the beginning of the disease, when the quantity of sugar is still very small, the urine often does not appear abnormal. In the more developed, typical cases the quantity of urine voided increases considerably, to 3–6–10 liters per day. The percentage of the physiological constituents is as a rule very low, while their absolute daily quantity is increased. The urine is pale, but of a high specific gravity, 1.030–1.040 or even higher. The high specific gravity depends upon the quantity of sugar present, which varies in different cases, but may reach 10 per cent. The urine is therefore characterized in typical cases of diabetes by the very large quantity voided, by the pale color and high specific gravity, and by its containing sugar.

That the urine after the introduction into the system of certain medicinal agents or poisonous bodies contains reducing substances, conjugated glucuronic acids, which may be mistaken for sugar, has already been mentioned.

The properties and reactions of dextrose have been considered in a previous chapter, and it remains but to mention the methods for the detection and quantitative determination of dextrose in the urine.

The *detection of sugar* in the urine is ordinarily, in the presence of not too small quantities, a very simple task. The presence of only very small quantities may make its detection sometimes very difficult and laborious. A urine containing proteid must first have the proteid removed by coagulation with acetic acid and heat before it can be tested for sugar.

The tests which are most frequently employed and are especially recommended are as follows:

TROMMER'S *Test.* In a typical diabetic urine or one rich in sugar this test succeeds well, and it may be performed in the manner suggested on page 207. This test may lead to very great mistakes in urines poor in sugar, especially when they have at the same time normal or increased amounts of physiological constituents, and therefore it cannot be recommended to physicians or to persons inexperienced in such work. Normal urine contains reducing substances, such as uric acid, creatinine, and others, and therefore a reduction takes place in all urines on using this test. A separation of copper suboxide does not generally occur, but still if one varies the proportion of the alkali to the copper sulphate and boils, there takes place an actual separation of suboxide in normal urines, or a peculiar

yellowish red liquid due to finely divided cuprous hydroxide. This occurs especially on the addition of much alkali or too much copper sulphate, and by careless manipulation the inexperienced worker may therefore sometimes obtain apparently positive results in a normal urine. On the other hand, as the urine contains substances, such as creatinine and ammonia (from the urea), which in the presence of only a little sugar may keep the copper suboxide in solution, the investigator may easily overlook small quantities of sugar that may be present.

The delicacy of TROMMER'S test can be increased by the suggestion made by WORM MÜLLER.[1] As by this rather complicated and tedious method small amounts of sugar cannot be detected in certain urines, and also as special urines from healthy persons readily give inconclusive results, and finally as SCHÖNDORFF has shown in numerous cases that the physiological sugar content of the urine responds to this test in perfectly healthy persons because of its extreme delicacy, it does not seem advisable in HAMMARSTEN'S opinion to recommend this test to the physician. BANG and BOHMANSSON[2] have recently also shown its unreliability.

ALMÉN'S *bismuth test*, which has been incorrectly called NYLANDER'S test, is performed with the alkaline-bismuth solution prepared as described on page 208. For each test 10 cc. of urine are taken and treated with 1 cc. of the bismuth solution and boiled for a few minutes. In the presence of sugar the urine becomes dark yellow or yellowish brown. Then it grows darker, cloudy, dark brown, or nearly black, and non-transparent. After a longer or shorter time a black deposit appears, the supernatant liquid gradually clears, but still remains colored. In the presence of only very little sugar the test does not become black or dark brown, but simply deeper-colored, and not until after some time is there seen on the upper layer of the phosphate precipitate a dark or black layer (of bismuth?). In the presence of much sugar a larger amount of the reagent may be used without disadvantage. In a urine poor in sugar only 1 cc. of the reagent for every 10 cc. of the urine must be employed.

Small amounts of proteid may retard this reaction and reduce the delicacy of the test. Large quantities of proteid may, however, give rise to an error by forming bismuth sulphide, and therefore it must always be first removed. The assertion of BECHHOLD that mercury compounds in the urine disturb the test has not been substantiated by ZEIDLITZ[3] on properly performing the test. Those sources of error which in TROMMER'S test are caused by the presence of uric acid and creatinine are removed by using this test. The bismuth test is, moreover, readily performed, and on this account is to be recommended to the physician.

The bumping and ejection of the fluid can be readily prevented by heating over a very small flame after the test has been brought to a boil, and by gently

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[1] In regard to this test see Pflüger, Pflüger's Arch., **105** and **106**; Hammarsten, *ibid.*, **116**, and Zeitschr. f. phyisol. Chem., **50**.

[2] Schöndorff, Pflüger's Arch., **121**; Bohmansson, Bioch. Zeitschr., **19**.

[3] Bechhold, Zeitschr. f. physiol. Chem., **46**; Zeidlitz, Upsala Läkaref. Förh. (N. F.), **11** (Hammarsten Festschr).

shaking the contents of the not too narrow test-tube. The recommendation of heating for a longer time in the water-bath, fifteen minutes or more, is to be discarded, as the delicacy of the test is thereby so much increased that it gives a reaction with a physiological sugar content of 0.02 per cent.

When the amount of sugar in the urine is not less than 0.1 per cent a positive reaction is obtained if the test is boiled for 2–3 minutes and then allowed to stand quietly for 5 minutes. The phosphate precipitate is then black or nearly black. In detecting smaller quantities of sugar —0.05 per cent, the test as a rule must be boiled longer—about 5 minutes.

The value of this test lies in the fact that it positively detects small quantities of sugar—0.1 per cent or somewhat less. Like TROMMER'S test it is a reduction test, and shows also certain other reducing bodies besides the sugar. These bodies are certain conjugated glucuronic acids which may appear in the urine. After the use of certain therapeutic agents, such as rhubarb, senna, antipyrine, salol, turpentine and others, the bismuth test gives positive results. From this it follows that we should never be satisfied with this test alone, especially when the reduction is not very great.

According to BOHMANSSON and BANG [1] this test is perfectly reliable if about 10 cc. of the urine is treated with one-fifth volume of 25 per cent HCl and about 1 vol. moist animal charcoal (or ½ vol. dry) and shaken for about one minute and then filtered. The filtrate on neutralization with a few cubic centimeters of caustic soda is used for the ALMÉN test. The disturbing reducing substances are removed by the animal charcoal, but the sugar is not.

Fermentation Test. On using this test the process must vary according as the bismuth test shows small or large quantities of sugar. If a rather strong reduction is obtained, the urine may be treated with yeast and the presence of sugar determined by the generation of carbon dioxide. In this case the acid urine, or that faintly acidified with a little sulphuric or hydrochloric acid, is treated with compressed yeast, or yeast which has previously been washed by decantation with water. Pour this urine to which the yeast has been added into a SCHRÖTTER'S gas-burette or a LOHNSTEIN'S saccharimeter (see below). As the fermentation proceeds, the carbon dioxide collects in the upper part of the tube, while a corresponding quantity of liquid is expelled below. As a control in this case two similar tests must be made, one with normal urine and yeast to learn the quantity of gas usually developed, and the other with a sugar solution and yeast to determine the activity of the yeast. According to VICTOROW [2] the fermentation is complete after six hours at a temperature of 34–36° C.

If, on the contrary, only a faint reduction with the bismuth test is found, no positive conclusion can be drawn from the absence of any carbon dioxide or the appearance of a very insignificant quantity. The urine absorbs considerable amounts of carbon dioxide, and in the presence of only small amounts of sugar the fermentation test as above performed

[1] Bioch. Zeitschr., **19**.

[2] Pflüger's Arch., **118**.

may lead to negative or inaccurate results. In this case proceed in the following way: Treat the acid urine, or urine which has been faintly acidified with a little sulphuric acid, with yeast whose activity has been tested by a special test on a sugar solution, and allow it to stand 24–30 hours at about 30°. Then test again with the bismuth test, and if the reaction now gives negative results, then sugar was previously present. But if the reaction continues to give positive results, then it shows, if the yeast is active, the presence of other reducing, unfermentable substances.

In performing the fermentation test care should be taken that the urine be acid before as well as after fermentation. If the reaction becomes alkaline during fermentation (alkaline fermentation), then the test must be discarded. The vessel must be perfectly clean and strongly heated before use. To make sure the urine may be boiled before fermentation.[1]

If a good polariscope is at hand it must not be forgotten to control the results of the fermentation by determining the rotation before and after fermentation. The phenylhydrazine test also, in many otherwise doubtful cases, gives good service in testing urines for sugar.

Phenylhydrazine Test. According to v. JAKSCH this test is performed in the following way: Add in a test-tube containing 6–8 cc. of the urine two knife-pointfuls of phenylhydrazine hydrochloride and three knife-pointfuls of sodium acetate, and when the salts do not dissolve on warming add more water. The test-tube is placed in boiling water and warmed on the water-bath. It is then placed in a beaker of cold water. If the quantity of sugar present is not too small, a yellow crystalline precipitate is now obtained. If the precipitate appears amorphous, there are found, on looking at it under the miscroscope, yellow needles singly and in groups. If very little sugar is present, pour the test into a conical glass and examine the sediment. In this case at least a few phenylglucosazone crystals are found, while the occurrence of larger and smaller yellow plates or highly refractive brown globules does not show the presence of sugar. This reaction is very reliable, and by it the presence of 0.03 per cent sugar can be detected (ROSENFELD, GEYER[2]). In doubtful cases where certainty is desired, prepare the crystals from a large quantity of urine, dissolve them on the filter by pouring over them hot alcohol, treat the filtrate with water, and boil off the alcohol. Still better, the precipitate is dissolved, according to NEUBERG, in some pyridine, and again precipitated as crystals by the addition of benzene, ligroin, or ether. If the characteristic yellow crystalline needles, whose melting-point (204–205° C.) may also be determined, are now obtained, then this test is decisive for the presence of sugar. It must not be forgotten that levulose gives the same osazone as dextrose, and that a further investigation is necessary in certain cases.

The following modification by A. NEUMANN is simple, practical, and at the same time sufficiently delicate. 5. cc. of the urine are treated with 2 cc. of acetic

[1] On the performance of the fermentation test and certain sources of error, see Salkowski, Berlin. klin. Wochenschr., 1905 (Ewald-Festnummer), and Pflüger, Pflüger's Arch., **105** and **111**.

[2] Rosenfeld, Deutsch. med. Wochenschr., 1888; Geyer, cited from Roos, Zeitschr. f. physiol. Chem., **15**.

acid (30-per cent) saturated with sodium acetate, 2 drops of pure phenylhydrazine added, and the mixture boiled in a test-tube until it measures 3 cc. After quickly cooling warm again and then allow it to cool slowly. After 5–10 minutes beautifully formed crystals are obtained even in the presence of only 0.02 per cent sugar. According to the experience of HAMMARSTEN this modification, even in the presence of 0.1 per cent sugar in concentrated urines, does not always give a positive reaction. SALKOWSKI [1] has suggested an even more simple method.

The value of the phenylhydrazine test has been considerably debated, and the objection has been made that glucuronic acids also give a similar precipitate. A confounding with glucuronic acid is, according to HIRSCHL, not to be apprehended when the test is heated in the water-bath for a long time (one hour). KISTERMANN found this precaution insufficient, and Roos states that the phenylhydrazine test always gives a positive result with human urine, which coincides with E. HOLMGREN'S [2] and HAMMARSTEN'S experience. This test only shows a non-physiological quantity of sugar when a rather abundant crystallization is obtained from a small quantity of urine (about 5 cc.). Too great a delicacy of test is not to be recommended.

RUBNER'S *test* is performed as follows: The urine is precipitated with an excess of a concentrated lead-acetate solution and the filtrate carefully treated with enough ammonia to produce a flocculent precipitate. It is then heated to boiling, when the precipitate becomes flesh-colored or pink in the presence of sugar.

Polarization. This test is of great value, especially as in many cases it quickly differentiates between dextrose and other reducing, sometimes levogyrate, substances, such as the conjugated glucuronic acids. In the presence of only very little sugar the value of this test depends on the delicacy of the instrument and the dexterity of the observer. As a urine which shows no rotation or is actually faintly levorotatory, may contain 0.2 per cent sugar or perhaps even more, this test must be combined with the fermentation test if we are seeking very small amounts of sugar. The sugar in these cases can be detected only by the use of a very accurate and delicate instrument. This method is in many cases not serviceable for the physician. If the urine is clarified and partly decolorized by precipitation with lead acetate it must be done in acid solution with acetic acid.[3]

In the isolation of sugar and carbohydrates from the urine the benzoic-acid esters of the same may be prepared according to BAUMANN'S method. The urine is made alkaline with caustic soda to precipitate the earthy phosphates, the filtrate treated with 10 cc. of benzoyl chloride and 120 cc. of 10 per cent caustic soda solution for every 100 cc. of the filtrate (REINBOLD [4]), and shaken until the odor of benzoyl chloride has disappeared. After standing sufficiently long the ester is collected, finely divided, and saponified with an alcoholic solution of sodium

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[1] Neumann, Arch. f. (Anat. u.) Physiol., 1899, Suppl. See also Margulies, Berlin. klin. Wochenschr., 1900; Salkowski, Arbeiter aus dem pathol. Inst., Berlin, 1906.

[2] Hirschl, Zeitschr. f. physiol. Chem., **14**; Kistermann, Deutsch. Arch. f. klin. Med., **50**; Roos, l. c.; Holmgren, Maly's Jahresber., **27**.

[3] See Grossmann, Bioch. Zeitschr., **1**.

[4] Pflüger's Arch., **91**.

ethylate in the cold according to BAISCH'S method,[1] and the various carbohydrates separated according to his suggestion.

If small quantities of sugar are to be isolated from the urine, precipitate the urine first with sugar of lead, filter, precipitate the filtrate with ammoniacal basic lead acetate, wash this precipitate with water, decompose it with H_2S when suspended in water and use the filtrate for the special tests. SCHÖNDORFF[2] has suggested a method for the detection and estimation of very small amounts of sugar based upon the work of PATEIN and DUFAU. This method depends upon precipitating the nitrogenous substances with mercuric nitrate.

To the physician, who naturally wants simple and quick methods, the bismuth test is especially to be recommended. If this test gives negative results, the urine is to be considered as free from sugar in a clinical sense. If it gives positive results, the presence of sugar must be controlled by other tests, especially by the fermentation test.

Other tests for sugar, as, for example, the reaction with orthonitrophenylpropiolic acid, picric acid, diazobenzene-sulphonic acid, are superfluous. The reaction with α-naphthol, which is a reaction for carbohydrates in general, for glucuronic acid and mucin, may, because of its extreme delicacy, give rise to mistakes, and is therefore not to be recommended to physicians. Normal urines give this test, and if the strongly diluted urine gives the reaction the presence of great quantities of carbohydrates may be suspected. In these cases more positive results are obtained by using other tests. This test requires great cleanliness, and it has the inconvenience that sufficiently pure sulphuric acid is not always readily procurable. Several investigators, such as V. UDRÁNSKY, LUTHER, ROOS and TREUPEL,[3] have investigated this test in regard to its applicability as an approximate test for carbohydrates in the urine.

Quantitative Determination of Sugar in the Urine. The quantity of sugar can be determined by *titration*, by *fermentation* of the sugar, by *polarization*, and also in other ways.

The titration methods are based upon the property of the sugar to reduce metallic oxides in alkaline solutions. As the titration liquids (cupric oxide solution in the FEHLING-SOXHLET, PAVY, BANG methods and mercuric oxide in KNAPP'S method) are also reduced by other urinary constituents, these reduction methods always give too high results. When large quantities of sugar are present, as in typical diabetic urine, which generally contains a lower percentage of normal reducing constituents, this is indeed of little account; but when small quantities of sugar are present in an otherwise normal urine, the mistake may, on the contrary, be important, as the reducing power of normal urine may correspond to 5 p. m. dextrose (see page 711). In such cases the titration procedure must be employed in connection with the fermentation method, which will be described later.

Of the titration methods with copper solutions the method suggested by BANG is the simplest, and at the same time seems to be more reliable than any of the others. For this reason we will describe only this method

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[1] Zeitschr. f. physiol. Chem., **19**.

[2] Pflüger's Arch., **121**, which cites the work of Patein and Dufau.

[3] See Roos and Treupel, Zeitschr. f. physiol. Chem., **15** and **16**.

and refer to the original works and to HOPPE-SEYLER-THIERFELDER, Handbuch der Chem. Analyses, 1909, for description of the titration of FEHLING'S solution according to SOXHLET [1] and to the titration according to PAVY and KUMAGAWA-SUTO.[2]

BANG'S *method*.[3] The principle of this method is that when urine is boiled with an excess of a solution of potassium carbonate, potassium thiocyanate and copper sulphate, copper thiocyanide is formed, and this remains in solution as a colorless compound. The excess of cupric oxide remaining is determined by titration with a solution of hydroxylamine until the blue color disappears. The quantity of sugar is calculated from the quantity of hydroxylamine used. The following solutions are necessary: (*a*) A copper salt solution containing 25 grams cupric sulphate in 2 liters; and (*b*) a solution containing 6.55 grams hydroxylamine sulphate in 2 liters.

The copper solution is prepared in the following manner: 500 grams potassium carbonate, 400 grams potassium thiocyanate and 100 grams potassium bicarbonate are dissolved in 1200 cc. water in a graduated flask and if necessary warmed to 50–60° C. On cooling to the ordinary temperature add very slowly 150 cc. of a cool, aqueous solution of cupric sulphate which contains 25 grams cupric sulphate ($CuSO_4+5H_2O$) in 150 cc.; then add water up to 2 liters. After standing at least 24 hours filter, and this solution can be kept indefinitely. The hydroxylamine solution is prepared by dissolving 200 grams potassium thiocyanate in about 1500 cc. water in a 2-liter graduated flask and adding a solution of 6.55 grams hydroxylamine sulphate in water; then add water to the 2-liter mark. This solution also keeps, but it must be kept in a dark-colored bottle. Equal volumes of each of these two solutions should exactly correspond to each other, and this can be determined by titrating at ordinary temperature 50 cc. of the copper solution (plus 10 cc. water) with the hydroxylamine solution.

The presence of proteid does not interfere with the reaction, and it is not necessary to remove the proteid. If more than 3 per cent sugar is suspected in the urine the latter must be diluted with a known amount of water. In the estimation 10 cc. of the fluid containing sugar is always used. If the urine contains less than 0.6 per cent sugar, then 10 cc. are used; otherwise, according to the amount of sugar, 5–2 cc. of the urine are diluted with water to 10 cc. and this used in the determination.

Performance of the determination. 10 cc. of the sugar fluid are placed in a glass flask and treated with 50 cc. of the copper solution. This is heated on a wire-gauze to boiling, boiled for three minutes, cooled quickly with water to the temperature of the room and then the hydroxylamine solution allowed to flow in from a burette until the blue color disappears and the solution is colorless, or, in urine poor in sugar, is yellow. This yellow coloration may disturb the end reaction somewhat, so that with inexperienced workers an error of 0.5 cc. hydroxylamine solution (corresponding to 0.5 milligram sugar) may be the result. If necessary the urine can be decolorized, according to ANDERSEN, by mercuric nitrate.

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[1] Journ. f. prakt. Chem., (N. F.), **21**.

[2] Pavy, The Physiology of the Carbohydrates, London, 1894; Kumagawa and Suto, Salkowski's Festschr., 1904; Sahli, Deutsch. med. Wochenschr., 1905.

[3] Bioch. Zeitschr., **2** and **11**. See also Funk, Zeitschr. f. physiol. chem., **56**; Jessen-Hansen, Bioch. Zeitschr., 10 and Andersen, *ibid.*, **15**.

The sugar in milligrams is directly obtained from the amount of hydroxylamine solution used by referring to the following reduction table:[1]

Hydroxylamine solution used.	Milligrams sugar	Hydroxylamine solution used.	Milligrams sugar.	Hydroxylamine solution used	Milligrams sugar.	Hydroxylamine. solution used.	Milligrams sugar.
0.75	60.0	13.00	39.0	25.50	23.5	38.00	10.4
1.00	59.4	13.50	38.3	26.00	22.9	38.50	9.9
1.50	58.4	14.00	37.7	26.50	22.3	39.00	9.4
2.00	57.3	14.50	37.1	27.00	21.8	39.50	9.0
2.50	56.2	15.00	36.4	27.50	21.2	40.00	8.5
3.00	55.0	15.50	35.8	28.00	20.7	40.50	8.1
3.50	54.3	16.00	35.1	28.50	20.1	41.00	7.6
4.00	53.4	16.50	34.5	29.00	19.6	41.50	7.2
4.50	52.6	17.00	33.9	29.50	19.1	42.00	6.7
5.00	51.6	17.50	33.3	30.00	18.6	42.50	6.3
5.50	50.7	18.00	32.6	30.50	18.0	43.00	5.8
6.00	49.8	18.50	32.0	31.00	17.5	43.50	5.4
6.50	48.9	19.00	31.4	31.50	17.0	44.00	4.9
7.00	48.0	19.50	30.8	32.00	16.5	44.50	4.5
7.50	47.2	20.00	30.2	32.50	15.9	45.00	4.1
8.00	46.3	20.50	29.6	33.00	15.4	45.50	3.7
8.50	45.5	21.00	29.0	33.50	14.9	46.00	3.3
9.00	44.7	21.50	28.3	34.00	14.4	46.50	2.9
9.50	44.0	22.00	27.7	34.50	13.9	47.00	2.5
10.00	43.3	22.50	27.1	35.00	13.4	47.50	2.1
10.50	42.5	23.00	26.5	35.50	12.9	48.00	1.7
11.00	41.8	23.50	25.8	36.00	12.4	48.50	1.3
11.50	41.1	24.00	25.2	36.50	11.9	49.00	0.9
12.00	40.4	24.50	24.6	37.00	11.4		
12.50	39.7	25.00	24.1	37.50	10.9		

For every $\frac{1}{10}$ cc. hydroxylamine solution used more than given in the table between 49.00–15.00, subtract 0.1 milligram from the corresponding sugar value and 0.2 milligram between 15.00–1.0.

For exact determinations of sugar the method as suggested by ALLIHN and modified by PFLÜGER[2] is the best suited.

The TITRATION ACCORDING TO KNAPP depends on the fact that mercuric cyanide in alkaline solution is reduced to metallic mercury by dextrose. The titration liquid should contain 10 grams of chemically pure dry mercuric cyanide and 100 cc. of caustic-soda solution of a specific gravity of 1.145 per liter. When the titration is performed as described below (according to WORM-MÜLLER and OTTO), 20 cc. of this solution should correspond to exactly 0.05 gram of dextrose. If the process is carried out in other ways, the value of the solution is different.

In this titration also, the quantity of sugar in the urine should be between ½ and 1 per cent, and the extent of dilution necessary be determined by a preliminary test. To determine the end-reaction as described below, the test for the excess of mercury is made with sulphuretted hydrogen.

In performing the titration allow 20 cc. of KNAPP'S solution to flow into a flask and dilute with 80 cc. of water, or when the urine contains less than 0.5 per cent of sugar use only 40–60 cc. After this heat to boiling and allow the diluted urine to flow gradually into the hot solution, at first 2 cc., then 1 cc., then 0.5 cc.,

[1] This table is given with the permission of the publisher, Julius Springer, Berlin.

[2] Pflüger's Arch., 66.

then 0.2 cc., and lastly 0.1 cc. After each addition let it boil ½ minute. When the end-reaction is approaching, the liquid begins to clarify and the mercury separates with the phosphates. The end-reaction is determined by taking a drop of the upper layer of the liquid into a capillary tube and then blowing it out on pure white filter-paper. The moist spot is first held over a bottle containing fuming hydrochloric acid and then over strong sulphuretted hydrogen. The presence of a minimum quantity of mercury salt in the liquid is shown by the spot becoming yellowish, which is best seen when it is compared with a second spot that has not been exposed to the gas. The end-reaction is still clearer when a small part of the liquid is filtered, acidified with acetic acid, and tested with sulphuretted hydrogen (Otto [1]). The calculations are just as simple as for the previous method.

This titration, unlike the previous one, may be performed equally well by daylight and by artificial light. Knapp's method has the following advantages over Fehling's method: It is applicable even when the quantity of sugar in the urine is very small and that of the other urinary constituents is normal. It is more easily performed, and the titration liquids may be kept without decomposing for a long time (Worm-Müller and his pupils [2]). There is diversity of opinion, nevertheless, among investigators on the value of this titration method.

Estimation of the Quantity of Sugar by Fermentation. This may be done in various ways; the simplest method, and one at the same time sufficiently exact for ordinary cases, is that of Roberts. This consists in determining the specific gravity of the urine before and after fermentation. In the fermentation of sugar, carbon dioxide and alcohol are formed as chief products and the specific gravity is lowered, partly on account of the disappearance of the sugar and partly on account of the production of alcohol. Roberts found that a decrease of 0.001 in the specific gravity corresponded to 0.23 per cent sugar, and this has been substantiated since by several other investigators (Worm-Müller and others). If the urine, for example, has a specific gravity of 1.030 before fermentation and 1.008 after, then the quantity of sugar contained therein was $22 \times 0.23 = 5.06$ per cent.

In performing this test the specific gravity must be taken at the same temperature before and after the fermentation. The urine must be faintly acid, and when necessary it should be acidified with a little hydrochloric acid or sulphuric acid. The activity of the yeast must, when necessary, be controlled by a special test. Place 200 cc. of the urine in a 400 cc. flask, add a piece of compressed yeast the size of a pea, and subdivide the yeast through the liquid by shaking; close the flask with a stopper provided with a finely-drawn-out glass tube, and allow the test to stand at the temperature of the room or, still better, at 30–35° C. After 24 hours the fermentation is ordinarily ended, but this must be verified by the bismuth test. After complete fermentation filter through a dry filter, bring the filtrate to the proper temperature, and determine the specific gravity.

If the specific gravity be determined with a good pyknometer supplied with a thermometer and an expansion-tube, this method, when the quantity of sugar is not less than 4–5 p. m., gives, according to Worm-Müller, very exact results, but this has been disputed by Budde.[3]

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[1] Journal f. prakt. Chem., **26**.

[2] Pflüger's Arch., **16** and **23**.

[3] Roberts, Edinburgh Med. Journ., 1861, and The Lancet, **1**, 1862; Worm-Müller,

For the physician the method in this form is not serviceable. Even when the specific gravity is determined by a delicate urinometer which can give the density to the fourth decimal, exact results are not obtained, because of the ordinary errors of the method (BUDDE); but the errors are usually smaller than those which occur in titrations made by unskilled hands.

When the quantity of sugar is less than 5 p. m. these methods cannot be used. Such small amounts cannot, as already mentioned, be determined by titration directly, because the reducing power of normal urine corresponds to 4–5 p. m. of sugar. In such cases, according to WORM-MÜLLER, it is better to first determine the reducing power of the urine by titration with KNAPP'S solution, then ferment the urine with the addition of yeast and titrate again with KNAPP'S solution. The difference found between the two titrations calculated as sugar gives the true quantity of the latter.

The determination of the sugar by fermentation can be so performed that the loss in weight due to the CO_2 can be estimated or the volume of the gas measured. For this last purpose LOHNSTEIN [1] has constructed a special fermentation saccharometer, and his "precision saccharometer" is to be recommended. Based upon LOHNSTEIN'S instrument, WAGNER [2] has constructed a "fermentation saccharo-manometer," which has certain advantages over LOHNSTEIN'S apparatus.

ESTIMATIONS OF SUGAR BY POLARIZATION. In this method the urine must be clear, not too deeply colored, and, above all, must not contain any other optically active substances besides dextrose. The urine may contain several levorotatory substances such as proteids, β-oxybutyric acid, conjugated glucuronic acids, the so-called LEO'S sugar and less often cystine, all of which are unfermentable. The proteid is removed by coagulation, and the others are detected by the polariscope after complete fermentation. The fermentable levulose is detected in a special manner (see below), and the dextrorotatory milk-sugar differs from dextrose in its not fermenting readily. By using a delicate instrument and with sufficient practice very exact results can be obtained by this method. The value of this procedure consists in the rapidity with which the determination can be made. In using instruments specially constructed for clinical purposes the accuracy is less than with the less expensive fermentation test. Under such circumstances, and as the estimation by means of polarization can be performed with exactitude only by specially trained chemists, it is hardly worth while to give this method in detail, and the reader is referred to handbooks for hints in the use of the apparatus.

Levulose. Levogyrate urines containing sugar have been noted by several investigators, although the nature of the sugar was not well known to the earlier observers. In recent years several positively authentic cases of levulosuria have been described, and also cases of diabetes

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Pflüger's Arch., **33** and **37**; Budde, *ibid.*, **40**, and Zeitschr. f. physiol. Chem., **13**. See also Huppert-Neubauer, 10. Aufl., and Lohnstein, Pflüger's Arch., **62**.

[1] Berlin. klin. Wochenschr., **35**, and Allg. med. Central-Ztg., 1899; Goldman, Chem. Centralbl., 1907, **1**, 1149.

[2] Münch. med. Wochensch., 1905.

have been found where levulose exists in the urine besides dextrose. Reports on this subject do not agree however.[1]

Levulose may be detected as follows: The urine is levorotatory and the levorotatory substance ferments with yeast. The urine gives the ordinary reduction tests and the ordinary phenylglucosazone. With methylphenylhydrazine it gives the characteristic levulose methylphenylosazone, and it also gives SELIWANOFF'S reaction on heating after the addition of an equal volume of hydrochloric acid and a little resorcin. With this test it must be remarked that too lengthy or too strong heating must not be applied, since other carbohydrates may also give the reaction (see page 211 and the works of ROSIN and UMBER[2]). After heating and cooling it can be neutralized with soda and shaken out with amyl alcohol, or with acetic ether (BORCHARDT). The amyl alcohol removes a red pigment which gives a band in the spectrum between *E* and *b* and on stronger concentration also a band in the blue at *F*. The acetic ether in the presence of levulose becomes yellow, and this is more characteristic according to BORCHARDT than ROSIN'S method, which has certain fallacies. The simultaneous presence of nitrites and indican disturbs the test, and in this case first remove the nitrous acid by boiling the urine, acidified with acetic acid, for one minute (BORCHARDT). In order to remove other disturbing pigments MALFATTI[3] suggests the oxidation of the urine with a little hydrochloric acid and potassium permanganate.

Maltose sometimes occurs in the urine according to Lepine and Boulud, and to Geelmuyden [4] and the latter has suggested a method of detecting maltose in the presence of dextrose by means of the fractional precipitation of the osazones.

Laiose is a substance named by HUPPERT and found by LEO [5] in diabetic urines in certain cases, and which he considers a sugar. It is levogyrate, amorphous, and does not taste sweet, but rather sharp and salty. Laiose has a reducing action on metallic oxides, does not ferment, and gives a non-crystalline, yellowish-brown oil with phenylhydrazine. There is no positive proof as yet that this substance is a sugar.

Lactose. The appearance of lactose in the urine of pregnant women was first shown by the observations of DE SINETY and F. HOFMEISTER, and this has been substantiated by other investigators. After the ingestion of large quantities of milk-sugar some lactose may be found in the urine (see Chapter IX on absorption). LANGSTEIN and STEINITZ have observed the passage of lactose and also of galactose [6] into the urine of nurslings with diseases of the stomach. The passage of lactose into the urine is called lactosuria.

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[1] See Borchardt, Zeitschr. f. physiol. Chem., 55, W. Voit, *ibid.*, 58.

[2] Umber, Salkowski's Festschrift, Berlin, 1904; Rosin, *ibid.*, and Zeitschr. f. physiol. Chem., 38.

[3] Rosin, l. c.; Borchardt, l. c.; Malfatti, Zeitschr. f. physiol. Chem., 58.

[4] Zeitschr. f. klin. Med., 63; Lépine and Boulud, Compt. rend., 132.

[5] Virchow's Arch., 107.

[6] Hofmeister, Zeitschr. f. physiol. Chem., 1, which also contains the pertinent literature. See also Lemaire, *ibid.*, 21; Langstein and Steinitz, Hofmeister's Beiträge, 7.

The positive detection of this sugar in the urine is difficult, because it is, like dextrose, dextrogyrate and also gives the usual reduction tests. If urine contains a dextrogyrate, non-fermentable sugar which reduces bismuth solutions, then it is very probable that it contains lactose. It must be remarked that the fermentation test for lactose is, according to the experience of LUSK and VOIT,[1] best performed by using pure cultivated yeast (saccharomyces apiculatus). This yeast only ferments the dextrose, while it does not decompose the milk-sugar. VOIT claims that if RUBNER'S test is performed without heating to boiling, but only to 80° C., the color becomes yellow or brown in the presence of lactose, instead of red. The most positive means for the detection of this sugar is to isolate the sugar from the urine. This may be done by the method suggested by F. HOFMEISTER.[2]

R. BAUER [3] detects galactose as well as lactose in the urine by oxidation with concentrated nitric acid, producing mucic acid.

Cammidge's reaction, which is recommended in the diagnosis of acute diseases of the pancreas, consists in that certain urines do not give the phenyl hydrazine reaction directly, but only after boiling with an acid. The reason of this is not known, but in a case examined by SMOLENSKI [4] the reaction to all appearances was due to cane-sugar.

Pentoses. SALKOWSKI and JASTROWITZ first found in the urine of persons addicted to the morphine habit a variety of sugar which was a pentose and yielded an osazone which melted at 159° C. Since this several other cases of pentosuria have been observed, and according to KÜLZ and VOGEL small amounts of pentose also occur in the urine of diabetics, as also in the urine of dogs with pancreatic or phlorhizin diabetes.[5]

The pentose isolated by NEUBERG from the urine in chronic pentosuria was *i*-arabinose. LUZZATTO has studied a case of pentosuria and found *l*-arabinose (see page 203). In alimentary pentosuria the *l*-arabinose of the plant food may be found in the urine. The appearance of pentoses in the urine after eating fruits and fruit-juices has been repeatedly observed by BLUMENTHAL and also by v. JAKSCH.[6]

A urine containing pentose reduces bismuth as well as copper solutions, although the reduction is not so rapid, but appears gradually. If only pentose is present, the urine does not ferment, but in the presence of dextrose small amounts of pentose may also undergo fermentation. The preparation of the osazone serves in the detection of pentoses;

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[1] Carl Voit, Ueber Die Glycogenbildung nach Aufnahme verschiedener Zuckeraten, Zeitschr. f. Biologie, 28.

[2] Hofmeister Zeitschr. f. physiol. Chem., **1**, which also contains the pertinent literature.

[3] Zeitschr. f. physiol. Chem., **51**.

[4] *Ibid.*, 60.

[5] In regard to the literature, see footnote 1, page 201. See also Blumenthal, "Die Pentosurie," Deutsche Klinik, 1902.

[6] Blumenthal, Deutsche Klinik, 1902; v. Jaksch, Centralbl. f. innere Medizin, 1906.

this compound when pure melts at 166–168° C., but when obtained from the urine has a melting-point of 156–160° C. The phloroglucin or orcin tests can also be employed (see page 202). Of these the last is most preferable, especially as it excludes a confusion with the conjugated glucuronic acids.

The orcin test can be performed as follows: 5 cc. of the urine is mixed with an equal volume of HCl sp.gr. 1.19, a small amount of orcin added and the whole heated to boiling. As soon as a greenish cloudiness appears cool the mixture off and shake carefully with amyl alcohol. The amyl-alcohol solution is used in the spectroscopic examination. The precipitation of a bluish-green pigment is in itself significant.

Bial [1] uses as reagent 30 per cent hydrochloric acid, which contains 1 gram of orcin and 25 drops of a ferric-chloride solution (62.9 per cent of the crystalline salt) in 500 cc. of the acid. 4.5 cc. of the reagent are heated to boiling and then a few drops (not more than 1 cc.) of the urine are added to the hot but not boiling liquid. In the presence of pentose the liquid turns a beautiful green. The usefulness of Bial's reagent is questioned by several experimenters. The delicacy is too great and the possibility of confounding with other carbohydrates is not excluded. In regard to the numerous modifications of this test and to Jolles reaction we refer to page 203. The same for the quantitative estimation of pentoses (page 202).

Rosenberger [2] believes he has detected a *heptose* in the urine in a case of diabetes. According to him and to Geelmuyden [3] probably different varieties of sugar, which are not well known, can possibly occur in urine of diabetics.

Conjugated Glucuronic Acids. Certain conjugated glucuronic acids such as menthol- and turpentine-glucuronic acid may spontaneously decompose in the urine, and in this case they may readily lead to a confusion with pentoses. The urine should always be fresh as possible for these examinations.

A confusion of the glucuronic acids, which have a reducing power on copper or bismuth solutions, with dextrose and levulose can be prevented by the fermentation test. They may also be distinguished from dextrose by their optical behavior, as the conjugated glucuronic acids are levogyrate. On boiling with an acid dextrorotatory glucuronic acid is produced and the levorotation is changed to dextrorotation.

The conjugated glucuronic acids, like the pentoses, give the phloroglucin-hydrochloric-acid test. On the contrary they do not give the orcin test directly, but only after cleavage with the setting free of glucuronic acid. On using Bial's reagent no mistaking for pentoses occurs, although this statement requires further substantiation. The pentoses may also

[1] Deutsch. med. Wochenschr., 1903; see also footnote 3, page 203.

[2] Zeitschr. f. physiol. Chem., **49**.

[3] Rosenberger, Centralbl. f. inn. Med., **28**; Geelmuyden, Zeitschr. f. klin. Med., **58** and **63**.

be isolated and identified by their osazones. The occurrence of conjugated glucuronic acid in the urine is shown when the urine does not give the orcin-hydrochloric-acid reaction directly, but only after boiling with the acid. The naphthoresorcin reaction, as suggested by TOLLENS,[1] can also be used. To 5 cc. urine add 0.5 cc. of a 1-per cent alcoholic solution of naphthoresorcin and 5 cc. hydrochloric acid (sp.gr. 1.19), boil for one minute, allow to stand four minutes, cool and shake with ether. In the presence of glucuronic acid the ether becomes violet or blue and shows the absorption bands given on page 216.

A further proof is that suggested by v. ALFTHAN;[2] 500 cc. of the urine is benzoylated and the ester obtained saponified with sodium ethylate. The free and conjugated glucuronic acid is thus obtained as sodium compounds, insoluble in alcohol, while the pentoses, if present, remain in the alcoholic filtrate. We have no sufficient experience as to the value of this method.

The surest method is that suggested by MAYER and NEUBERG, which consists in precipitating the urine with basic lead acetate, decomposing the precipitate with H_2S, boiling with dilute sulphuric acid in order to split the conjugated acid, and then after neutralizing with soda preparing the characteristic bromphenylhydrazine compound of glucuronic acid (see page 216) with *p*-bromphenylhydrazine hydrochloride and sodium acetate. HERVIEUX[3] has slightly modified this method.

Inosite seems to be a normal urinary constituent, although it occurs only in very small quantities (HOPPE-SEYLER, STARKENSTEIN[4]). In diabetes insipidus, as well as after excessive drinking of water, it occurs in large quantities in the urine because of a more abundant washing-out of the tissues.

For the detection of inosite we make use of the method given on page 552, with the modifications suggested by MEILLÈRE and STARKENSTEIN.

Acetone Bodies (acetone, acetoacetic acid, β-oxybutyric acid). These bodies, whose occurrence in the urine and formation in the organism have been the subject of numerous investigations, occur in the urine especially in diabetes mellitus, but also in many other diseases.[5] According to v. JAKSCH and others, acetone is a normal urinary constituent, though it may occur only in very small amounts (0.01 gram in twenty-four hours).

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[1] Ber. d. d. chem. Gesellsch., **41**, 1788, and C. Tollens, Zeitschr. f. physiol. Chem., **56**. See also Mandel and Neuberg, Bioch. Zeitschr., **13**.

[2] Arch. f. exp. Path. u. Pharm., **47**.

[3] Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Hervieux, Compt. rend. soc. biol., **63**.

[4] Starkenstein, Zeitschr. f. exp. Path. u. Therap., **5**, which contains the literature.

[5] In regard to the extensive literature on acetone bodies the reader is referred to Huppert-Neubauer, Harn-Analyse, 10. Aufl., and v. Noorden's Lehrb. d. Pathol. des

In regard to the origin of these bodies it was formerly considered that they were produced by an increased destruction of protein. One of the various reasons for this was the increase in the elimination of acetone and acetoacetic acid during inanition (v. Jaksch, Fr. Müller[1]). This also stands in accord with the observations that a considerable increase in the quantity of acetone and acetoacetic acid eliminated is observed in such diseases as fevers, diabetes, digestive disturbances, mental diseases with abstinence and cachexia, where the body protein is largely destroyed. The formation of acetone bodies from protein is also indicated by the fact that acetone has been obtained as an oxidation product from gelatin and protein (Blumenthal and Neuberg, Orgler[2]). The investigations of Embden and collaborators are more conclusive. After Embden and Kalberlah showed that the liver is an organ where acetone is formed, Embden, Salomon and Schmidt[3] showed by experiments on extirpated livers, that butyric acid, oxybutyric acid, leucine, tyrosine and in fact those aromatic bodies which, like tyrosine, phenylalanine, phenyl-α-lactic acid and homogentisic acid contain a combustible benzene nucleus, are transformed, in the liver, into acetone. Research, which has been continued further by Embden and his collaborators and substantiated by others, such as Baer, Blum, Borchardt and Lange,[4] has shown that there can be no doubt that leucine in particular is a strong acetone former, and consequently that acetone can be formed from protein. Protamines and histones can also increase the acetone elimination (Borchardt) or, as we say may have a "ketoplastic" action, and it is therefore possible that acetone can be formed from arginine with α-amino-valerianic acid as intermediary step (Borchardt and Lange).

As we cannot deny the possibility of a formation of acetone from proteins, on the other hand we have observations which are inconsistent with the origin of the acetone bodies entirely from the proteins. Thus no parallelism exists between the acetone bodies and the nitrogen excretion in diabetics, and the fact that in man no certain relation exists between the acetone elimination and the nitrogen and sulphur excretion seems to

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Stoffwechsels. Berlin, 1906, and for recent work, Magnus-Levy, Die Azetonkörper, Ergbn. d. inn. Med. u. Kinderheilk., I.

[1] v. Jaksch, Ueber Acetonurie und Diaceturie. Berlin, 1885; Fr. Müller, Bericht über die Ergebnisse des an Cetti ausgeführten Hungerversuches. Berlin. klin. Wochenschr., 1887.

[2] Blumenthal and Neuberg. Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, 1.

[3] Hofmeister's Beiträge, 8.

[4] Embden, *ibid.*, 11, with Marx, Engel, Lattes and Michaud, *ibid.*, 11; Baer and Blum, Arch. f. exp. Path. u. Pharm., 55 and 56; Borchardt, *ibid.*, 53, with Lange, Hofmeister's Beiträge, 9.

show that the acetone bodies are not entirely derived from the proteins. In man the excretion of acetone does not increase with the rise in the quantity of protein, and an increase in the latter above the average causes a diminution in the elimination of acetone (ROSENFELD, HIRSCHFELD, FR. VOIT [1]). The carbohydrates cannot be considered as material for the formation of acetone bodies. It is generally admitted that in man the exclusion of carbohydrates from the food or the diminution in their amount or their assimilation may lead to more or less increased elimination of acetone bodies. This behavior may occur in diabetes as well as in starvation and in the above-mentioned diseased conditions. The increased elimination of acetone with food lacking carbohydrates also occurs in healthy persons with a fatty diet but with a sufficient supply of calories in other ways (alimentary acetonuria). With an abundant supply of carbohydrates the elimination of acetone bodies may be greatly diminished or even stopped entirely. The carbohydrates therefore act "antiketoplastic," and a similar retarding action can be produced by certain other substances, such as glycerin (HIRSCHFELD), tartaric acid, lactic acid and citric acid (SATTA), alanine and asparagin (BORCHARDT and LANGE [2]). Certain bodies like glycerin, lactic acid, alanine, asparagin, which cause a sugar formation or increased elimination of sugar, act in the same way.

It must not be overlooked that the conditions are different in man and in other carnivora (GEELMUYDEN, FR. VOIT). In dogs the elimination of acetone bodies is not increased in starvation, but is reduced; it is augmented with increased quantities of meat, runs parallel with the nitrogen excretion, and is not diminished by carbohydrates (FR. VOIT [3]). In spite of this divergent behavior an unmistakable relation also exists in the dog between the elimination of acetone bodies and the carbohydrate metabolism, because in phlorhizin diabetes the *acidosis* occurs only after the glycogen has been consumed (MARUM [4]).

As the carbohydrates cannot be acetone-formers, then a second source only remains, namely the fats. As proof of this there are certain cases of diabetes with strong elimination of acetone bodies (β-oxybutyric acid) where the quantity of protein transformed was too small to account for the acetone bodies (MAGNUS-LEVY). The free elimination of acetone bodies in starvation may also depend upon the fact that a great part of the body fat is consumed, and in several cases a certain relation has

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[1] Hirschfeld, Zeitschr. f. klin. Med., 28; Geelmuyden, see Maly's Jahresber., 26, and Zeitschr. f. physiol. Chem., 23 and 26; Rosenfeld, Centralbl. f. innere Med., 16; Voit, Deutsch. Arch. f. klin. Med., 66.

[2] l. c. Hofmeister's Beiträge, 9, which also cites the other works.

[3] See footnote 1.

[4] Hofmeister's Beiträge, 10.

been found between the fat consumed and the acetone bodies eliminated. Certain investigators (GEELMUYDEN, SCHWARZ, WALDVOGEL [1]) have also observed an increase in the acetonuria on partaking of fatty food, and at the present time the fats are considered as the most important source of the acetone bodies.

The three acetone bodies occurring in the urine, as above stated, are acetone, acetoacetic acid and β-oxybutyric acid, and this last is considered as the mother-substance of the other two. If β-oxybutyric acid, $CH_3.CHOH.CH_2.COOH$, is introduced into the animal body, it is burnt if the quantity is not too great, while if in excess it passes into the urine as acetoacetic acid, $CH_3.CO.CH_2.COOH$. This acid can also be burnt, but if large quantities are introduced it appears in part in the urine and readily splits into acetone, $CH_3.CO.CH_3$, and CO_2. Acetone is in part burnt in the animal body, but a part is eliminated by the kidneys and especially by the lungs. We can imagine that the β-oxybutyric acid is a physiological metabolic product which normally is completely changed into acetoacetic acid and acetone, and in diabetes and especially with lack of carbohydrates is formed to an increased extent, or its combustion made more difficult, so that in the first place acetone and acetoacetic acid pass into the urine and in severe cases also β-oxybutyric acid (acidosis).

That acetone bodies can be formed from proteins is shown by the perfusion experiments of EMBDEN and ENGEL with livers and leucine, where acetone is formed in the liver with acetoacetic acid as intermediary body. It is also probable, in correspondence with the observations of BAER and BLUM,[2] that in diabetics leucine and isovalerianic acid cause an increase in the elimination of β-oxybutyric acid, and that β-oxybutyric acid is formed from leucine, with isovaleric acid, $\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH.CH_2COOH$, as intermediary step. In the formation of acetone from tyrosine and phenylalanine, acetoacetic acid is formed as an intermediary, and probably also β-oxybutyric acid (EMBDEN and ENGEL).

In regard to the formation of acetone bodies from fat it must be remarked that glycerin has an antiketoplastic action and that the fatty acids can only be considered. As to the behavior of these in the formation of acetone, EMBDEN and MARX [3] have shown that only those normal fatty acids which contain an even number of carbon atoms are acetone formers, while those with an uneven number of carbon atoms

[1] Magnus-Levy, Arch. f. exp. Path. u. Pharm., **42**; Geelmuyden, l. c., and Norsk, Magazin for Laegevidenskaben, 1900, see also Zeitschr. f. physiol. Chem., **41**, Schwarz, Deutsch. Arch. f. klin. Med., 1903; Waldvogel, Centralbl. f. innere Med., **20**.

[2] Arch. f. exp. Path. u. Pharm., **55** and **56**; Embden and Engel, Hofmeister's Beiträge, **11**.

[3] Hofmeister's Beiträge, **11**.

are without action in this regard. This is true at least for the acids from *n*-decanoic acid to *n*-butyric acid, which latter is a strong acetone former. As in diabetics a greater number of oxybutyric acid molecules can be eliminated than corresponds to the number of fatty acid molecules decomposed, it seems as if more than one molecule of β-oxybutyric acid is produced from one molecule of fatty acid. We cannot therefore admit of a simple demolition of the fatty acids to butyric acid (by consecutive oxidation attacks in the β-position) but rather a destruction of the fatty acid molecules into several parts, and which take part in the formation of β-oxybutyric acid. A synthetical formation of β-oxybutyric acid has been accomplished by GEELMUYDEN and others, but especially by MAGNUS-LEVY, starting with acetaldehyde, according to the hypothesis of SPIRO. It is also interesting that FRIEDMANN [1] has shown by perfusion experiments with livers that aldehyde ammonia, and to a greater extent aldol, are acetone formers. It must therefore be admitted that first a condensation of the aldehyde to aldol takes place, $CH_3.COH + CH_3.COH = CH_3.CH(OH).CH_2.COH$, and that β-oxybutyric acid, $CH_3.CH(OH).CH_2.COOH$, is formed from this by oxidation.

According to the above-mentioned perfusion experiments it must be admitted that the liver is important in the formation of acetone bodies, and EMBDEN and LATTES have found that the ability of the liver of the dog with pancreas diabetes or phloridzin diabetes to produce acetone is much greater than the liver of the normal animal. On the other hand, as shown by EMBDEN and MICHAUD,[2] in dogs and oxen the liver also has a strong destructive action upon acetoacetic acid. A similar action is also found in the kidneys, muscles and spleen of dogs and pigs. The destructive action of fresh organs is much stronger upon acetoacetic acid than upon acetone.

Acetone, C_3H_6O, dimethylketone, $CH_3.CO.CH_3$, is a thin, water-clear liquid, boiling at 56.3° and possessing a pleasant odor of fruit, which in diabetes gives a pomaceous or fruit odor to the urine as well as the expired air. It is lighter than water, with which it mixes in all proportions, also with alcohol and ether. The most important reactions for acetone are the following:

LIEBEN'S *Iodoform Test.* When a watery solution of acetone is treated with alkali and then with some iodo-potassium-iodide solution and gently warmed, a yellow precipitate of iodoform is produced, which is known by its odor and by the appearance of the crystals (six-sided plates or stars) under the microscope. This reaction is very delicate, but it is not characteristic of acetone. GUNNING'S *modification of the iodoform test* con-

[1] Geelmuyden, Zeitschr. f. physiol. Chem., **23** and **26**; Magnus-Levy, Arch. f. exp. Path. u. Pharm., **42**; Friedmann, Hofmeister's Beiträge, **11**.

[2] Embden and Lattes, Hofmeister's Beiträge, **11**; Embden and Michaud, *ibid.*, **11**.

sists in using an alcoholic solution of iodine and ammonia instead of the iodine dissolved in potassium iodide and alkali hydroxide. In this case, besides iodoform, a black precipitate of nitrogen iodide is formed, but this gradually disappears on standing, leaving the iodoform visible. This modification has the advantage that it does not give any iodoform with alcohol or aldehyde. On the other hand, it is not quite so delicate, but still it detects 0.01 milligram of acetone in 1 cc.

Reynold's *mercuric-oxide test* is based on the power of acetone to dissolve freshly precipitated HgO. A mercuric-chloride solution is precipitated by alcoholic caustic potash. To this add the liquid to be tested, shake well, and filter. In the presence of acetone the filtrate contains mercury, which may be detected by ammonium sulphide. This test has about the same delicacy as Gunning's test. Aldehydes also dissolve appreciable quantities of mercuric oxide.

Legal's *Sodium Nitroprusside Test.* If an acetone solution is treated with a few drops of a freshly prepared sodium-nitroprusside solution and then with caustic-potash or soda solution, the liquid is colored ruby-red. Creatinine gives the same color; but if the mixture is saturated with acetic acid, the color becomes carmine or purplish red in the presence of acetone, but yellow and then gradually green and blue in the presence of creatinine. With this test paracresol responds with a reddish-yellow color, which becomes light pink when acidified with acetic acid and cannot be mistaken for acetone. Rothera [1] has suggested a modification which is more delicate by using ammonium salts and ammonia.

Penzoldt's *indigo test* depends on the fact that orthonitrobenzaldehyde in alkaline solution with acetone yields indigo. A warm saturated and then cooled solution of the aldehyde is treated with the liquid to be tested for acetone and next with caustic soda. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates; and this may be dissolved with a blue color by shaking with chloroform; 1.6 milligrams acetone can be detected by this test.

Bêla v. Bitto's [2] reaction is based on the fact that on adding a solution of metadinitrobenzene, made alkaline with caustic potash, to acetone, a violet-red color is produced which becomes cherry-red on acidifying with an organic acid or metaphosphoric acid. Aldehyde gives a similar violet-red color which becomes yellowish-red on acidification. Creatinine does not give this reaction. Frommer [3] has suggested the following method for detecting acetone: Treat 10 cc. of the urine with 1 gram potassium hydroxide and add 10–20 drops of an alkaline solution of salicyl-aldehyde. On warming a purple-red coloration is obtained in the presence of acetone.

Acetoacetic acid, $C_4H_6O_3$, acetylacetic acid, diacetic acid, $CH_3.CO.CH_2.COOH$, is a colorless, strongly acid liquid which mixes with water,

[1] Journ. of Physiol., 37.

[2] Annal. d. Chem. u. Pharm., 239.

[3] Berlin. klin. Wochenschr., 1905.

alcohol, and ether in all proportions. On heating to boiling with water, and especially with acids, it decomposes into carbon dioxide and acetone, and therefore gives the above-mentioned reactions for acetone. It differs from acetone in that it gives a violet-red or brownish-red color with a dilute ferric-chloride solution. For the detection of this acid we make use of the following reactions which may be applied directly to the urine:

Gerhardt's *Reaction.* Treat 10–15 cc. of the urine with ferric-chloride solution until it fails to give a precipitate, filter, and add some more ferric chloride. In the presence of acetoacetic acid a wine-red color is obtained. The color becomes paler at the room temperature within twenty-four hours, but more quickly on boiling (differing from salicylic acid, phenol, sulphocyanides). A portion of the urine slightly acidified and boiled does not give this reaction on account of the decomposition of the acetoacetic acid.

Arnold *and* Lipliawsky's *Reaction.* 6 cc. of a solution containing 1 gram of *p*-aminoacetophenone and 2 cc. of concentrated hydrochloric acid in 100 cc. of water are mixed with 3 cc. of a 1-per cent potassium-nitrite solution and then treated with an equal volume of urine. A few drops of concentrated ammonia are now added and violently shaken. A brick-red coloration is obtained. Then take 10 drops to 2 cc. of this mixture (according to the quantity of acetoacetic acid in the urine), add 15–20 cc. HCl of sp.gr. 1.19, 3 cc. of chloroform, and 2–4 drops of ferric-chloride solution and mix without shaking. In the presence of acetoacetic acid the chloroform is colored violet or blue (otherwise only yellowish or faintly red). This reaction is more delicate than the preceding test and reacts with 0.04 p.m. acetoacetic acid. Large amounts of acetone (but not the quantity occurring in urines) give this reaction according to Allard.[1]

Bondi and Schwarz's[2] *Reaction.* 5 cc. of the urine is treated drop by drop with iodine-potassium iodide solution until the color is orange-red. Then warm gently and when the orange-red color has disappeared add the iodine solution again until the color remains permanent on warming. Then boil, when the irritating vapors of iodo-acetone will attack the eyes. Acetone does not give this reaction.

Detection of Acetone and Acetoacetic Acid in the Urine. Before testing for acetone test for acetoacetic acid; as this acid gradually decomposes on allowing the urine to stand, the specimen must be as fresh as possible. In the presence of acetoacetic acid the urine gives the above-mentioned tests. In testing for acetone in the presence of acetoacetic

[1] Arnold, Wien. klin. Wochenschr., 1899, and Centralbl. f. innere Med., 1900; Lipliawsky, Deutsch. med. Wochenschr., 1901; Allard, Berl. klin. Wochenschr., 1901.

[2] Wien. klin. Wochenschr., 1906.

acid make the urine slightly alkaline and shake in a separatory funnel with ether free from alcohol and acetone. Remove the ether and shake it with water, which takes up the acetone, and test for acetone in the watery solution.

In the absence of acetoacetic acid the acetone may be tested for directly in the urine; this may be done by PENZOLDT'S test or LEGAL'S test. These tests, which are only approximate, are of value only when the urine contains a considerable amount of acetone.

For a more accurate test we distill at least 250 cc. of the urine faintly acidified with sulphuric acid, care being taken to have a good condensation. Most of the acetone is contained in the first 10–20 cc. of the distillate. A better result may be obtained by distilling a large quantity of urine until about $\frac{1}{10}$ has been distilled off, acidify the distillate with hydrochloric acid, redistill and repeat this several times, collecting the first portion of each distillation. The final distillate is used for the above reactions.[1] SALKOWSKI and BORCHARDT have called attention to the fact that in the distillation of an acidified urine containing sugar for the detection or estimation of acetone a substance giving iodoform can be formed from the sugar if the distillation is carried too far. According to BORCHARDT [2] the urine must therefore first be diluted with water or the concentration prevented by the addition of water dropwise during distillation.

The *quantitative estimation* of acetone (also that formed from the acetoacetic acid) is done by distilling the urine after the addition of acetic acid or a little sulphuric acid. The quantity of acetone in the distillate can be determined, according to the HUPPERT-MESSINGER method, by converting it into iodoform by means of potassium iodide and then titrating the quantity of iodine used in the formation of the iodoform. The precipitation of the acetone as *p*-nitrophenylhydrazone-acetone by means of *p*-nitrophenylhydrazine in acetic acid solution can also be used for determining the acetone in the distillate (v. EKENSTEIN and BLANKSMA and MÖLLER). In regard to these methods we refer to[3]; EMBDEN, and SCHLIEP and FOLIN [4] have suggested methods for determining the quantity of acetone and acetoacetic acid separately.

β-Oxybutyric Acid, $C_4H_8O_3$, $CH_3.CH(OH).CH_2.COOH$, ordinarily forms an odorless syrup, but may also be obtained as crystals. It is readily soluble in water, alcohol, and ether. It is levorotatory; $(\alpha)_D = -24.12°$ for solutions of 1–11 per cent and has a disturbing action upon the determination of sugar by means of the polariscope. It is not precipitated by basic lead acetate or by ammoniacal lead acetate, neither does it ferment. On boiling with water, especially in the presence of a mineral acid, this acid decomposes into *α-crotonic acid,* which melts at 71–72° C., and water, $CH_3.CH(OH).CH_2.COOH = H_2O + CH_3.CH:CH.COOH$. It yields acetone on oxidation with a chromic-acid mixture.

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[1] See also Salkowski, Pflüger's Arch., **56**.

[2] Hofmeister's Beiträge, 8.

[3] Hoppe-Seyler, Thierfelder, 8. Aufl., 617 and 618.

[4] Embden and Schliep, Centralbl. f. d. ges. Phys. u. Path. d. Stoffwechsel, 1907; Folin, Journ. of biol. Chem., **3**.

Detection of β-Oxybutyric Acid in the Urine. If a urine is still levogyrate after fermentation with yeast, the presence of oxybutyric acid is probable. A further test may be made, according to KÜLZ, by evaporating the fermented urine to a syrup and, after the addition of an equal volume of concentrated sulphuric acid, distilling directly without cooling. α-crotonic acid is produced, which distills over, and, after collecting in a test-tube, crystals which melt at +72° C. separate on cooling. If no crystals are obtained, shake the distillate with ether, evaporate, and test the melting-point of the residue which has been washed with water. According to MINKOWSKI the acid may be isolated as a silver salt.[1]

The quantitative estimation is done by complete extraction of the β-oxybutyric acid by ether and determining the specific rotation. The extraction can be done according to MAGNUS-LEVY[2] or according to BERGELL.[3] Other methods of estimating β-oxybutyric acid have been suggested by DARMSTÄDTER, BOEKELMAN and BOUMA.[4]

EHRLICH'S[5] *Urine Test.* Mix 250 cc. of a solution which contains 50 cc. of HCl and 1 gram of sulphanilic acid is one liter with 5 cc. of a ½ per cent solution of sodium nitrite (which produces very little of the active body, sulphodiazobenzene). In performing this test treat the urine with an equal volume of this mixture and then supersaturate with ammonia. Normal urine will become yellow or orange after the addition of ammonia (aromatic oxyacids may after a certain time give red azo bodies which color the upper layer of the phosphate sediment). In pathological urines there sometimes occurs (and this is the characteristic diazo reaction) a primary yellow coloration, with a very marked secondary red coloration on the addition of ammonia, and the froth is also tinged with red. The upper layer of the sediment becomes greenish. The body which gives this reaction is unknown, but it especially occurs in the urine of typhoid patients (EHRLICH). Opinions differ in regard to the significance of this reaction. If the urine is made alkaline with sodium carbonate instead of ammonia and treated with a freshly prepared solution of diazobenzene sulphonic acid made alkaline with sodium carbonate, normal urine also gives an orange or Bordeaux-red coloration. The known normal urinary constituents which give the diazo reaction are the aromatic oxyacids, antoxyproteic acid and the imidazole derivative found by ENGELAND (see page 719).

Another urine test suggested by EHRLICH consists in adding hydrochloric acid containing 2 per cent dimethylaminobenzaldehyde to the urine; normal urines are colored faintly red, while certain pathological urines become cherry-red. The cause of this reaction is not sufficiently known; according to NEUBAUER it appears to be connected with the urobilinogen. HERTER[6] found that it was increased by a meat diet.

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[1] Arch. f. exp. Path. u. Pharm., **18**, 35; Zeitschr. f. anal. Chem., **24**, 153.

[2] See Hoppe-Seyler, Thierfelder's Handbuch, 8. Aufl., 619, and Geelmuyden, Hammarsten's Festschr., 1906.

[3] Zeitschr. f. physiol. Chem., **33**.

[4] Darmstädter, *ibid.*, **37**; Boekelman and Bouma, see Mary's Jahresber , **31**.

[5] Ehrlich, Zeitschr. f. klin. Med., **5**. See also Clemens, Deutsch. Arch. f. klin. Med., **63** (literature). Kutscher and Engeland, footnote 1, page 719.

[6] See Pröscher, Zeitschr. f. physiol. Chem., **31**, and Clemens, Deutsch. Arch. f klin. Med., **71**; Neubauer, Centralbl. f. Physiol., **19**, 145; HERTER, Journ. of biol. Chem.. **4**.

ROSENBACH'S urine test, which consists in adding nitric acid drop by drop to the boiling-hot urine and obtaining a claret-red coloration and a bluish-red foam on shaking, depends upon the formation of indigo substances, especially indigo-red.[1]

Fat in the Urine. The elimination of a urine which in appearance and richness in fat resembles chyle is called *chyluria*. It habitually contains a proteid and often fibrin. Chyluria occurs mostly in the inhabitants of the tropics. *Lipuria*, or the elimination of fat with the urine, may appear in apparently healthy persons, sometimes with and sometimes without albuminuria, in pregnancy, and also in certain diseases, as in diabetes, poisoning with phosphorus, and fatty degeneration of the kidneys.

Fat is usually detected by the microscope. It may also be dissolved with ether, and may invariably be detected by evaporating the urine to dryness and extracting the residue with ether.

Cholesterin is also sometimes found in the urine in chyluria and in a few other cases.

Amino-acids. Leucine and tyrosine have been repeatedly found in urine by the older methods, especially in acute yellow atrophy of the liver, in acute phosphorus poisoning, and in severe cases of typhoid and smallpox. Since β-naphthalene sulphochloride has been used in the detection of amino-acids these bodies have not only been repeatedly found in normal urine (glycocoll, see page 717), but also in pathological urines.[2]

Cystine (see page 146). BAUMANN and GOLDMANN[3] claim that a substance similar to cystine occurs in very small amounts in normal urine. This substance occurs in large quantities in the urine of dogs after poisoning with phosphorus. Cystine itself is only found with positiveness, and even then very rarely, in urinary calculi and in pathological urines, from which it may separate as a sediment. Cystinuria occurs oftener in men than in women. BAUMANN and V. UDRÁNSZKY found in urine in cystinuria the two diamines, *cadaverine* (pentamethylendiamine) and *putrescine* (tetramethylendiamine), which are produced in the putrefaction of proteins. These two diamines were also found in the contents of the intestine in cystinuria, while under normal conditions they are not present. HAMMARSTEN therefore considers that perhaps some connection exists between the formation of diamines in the intestine, by the peculiar putrefaction in cystinuria, and cystinuria itself. This is less probable, and cystinuria is, as generally admitted, rather an anomaly in the protein metabolism where the cystine for unknown reasons is not destroyed as ordinarily. It is remarkable that the cystine of the food-proteins is eliminated by the urine while in

[1] See Rosin, Virchow's Arch., **123**.

[2] Ignatowski, Zeitschr. f. physiol. Chem., **42**; Abderhalden and Schittenhelm, *ibid.*, **45**; Abderhalden and Barker, *ibid.*, **42**. See also footnote 5, page 717.

[3] Baumann, Zeitschr. f. physiol. Chem., **8**. In regard to the literature on cystinuria see Brenzinger, *ibid.*, **16**; Baumann and Goldmann, *ibid.*, **12**; Baumann and v. Udránszky, *ibid.*, **13**; Stadthagen and Brieger, Berlin. klin. Wochenschr., 1889; Cammidge and Garrod, Journ. of Path. and Bacteriol., 1900 (literature on diamines in the urine and feces); Loewy and Neuberg, Bioch. Zeitschr., **2**; Wolf and Schaffer, Journ. of biol. Chem., **4**.

cystinurics, at least sometimes, such cystine introduced is quantitatively transformed.[1] Cases of cystinuria may occur with or without the occurrence of diamines in the urine, and only rarely are the diamines found in the urine as well as in the feces, which perhaps depends upon the fact, as found by CAMMIDGE and GARROD[2] in one case, that the diamines occur only from time to time in the feces. The properties and reactions of cystine have been given on pages 147 and 148.

Cystine is easily prepared from cystine calculi by dissolving them in alkali carbonate, precipitating the solution with acetic acid, and redissolving the precipitate in ammonia. The cystine crystallizes on the spontaneous evaporation of the ammonia. The cystine dissolved in the urine is detected, in the absence of proteid and sulphuretted hydrogen, by boiling with alkali and testing with a lead salt or sodium nitroprusside. To isolate cystine from the urine, acidify the urine strongly with acetic acid. The precipitate containing cystine is collected after twenty-four hours and digested with hydrochloric acid, which dissolves the cystine and calcium oxalate, leaving the uric acid undissolved. Filter, supersaturate the filtrate with ammonium carbonate, and treat the precipitate with ammonia, which dissolves the cystine and leaves the calcium oxalate. Filter again and precipitate with acetic acid. The precipitated cystine is identified by the microscope and the above-mentioned reactions. Cystine as a sediment is identified by the microscope. It must be purified by dissolving in ammonia and precipitating with acetic acid; it is then further tested. Traces of dissolved cystine may be detected by the production of benzoyl-cystine, according to BAUMANN and GOLDMAN. For the detection and estimation of cystine we can proceed to advantage in the following manner, suggested by GASKELL[3]: The urine freed from oxalates and phosphates by means of ammonia and calcium chloride is treated with an equal volume of acetone and with acetic acid. The crystals which precipitate are dissolved in ammonia and then purified by reprecipitation with acetone.

VII. URINARY SEDIMENTS AND CALCULI.

Urinary sediment is the more or less abundant deposit which is found in the urine after standing. This deposit may consist partly of organized and partly of non-organized constituents. The first, consisting of cells of various kinds, yeast-fungi, bacteria, spermatozoa, casts, etc., must be investigated by means of the microscope, and the following only applies to the non-organized deposits.

As previously mentioned (page 639), the urine of healthy individuals may sometimes, even on voiding, be cloudy on account of the phosphates present, or become so after a little while because of the separation of urates. As a rule, urine just voided is clear, and after cooling shows

[1] See Wolf and Schaffer, Journ. of biol. Chem., 4.

[2] l. c. footnote 2, page 780.

[3] Journ. of Physiol., 36.

only a faint cloud (nubecula) which consists of urine mucoid, a few epithelium-cells, mucous corpuscles, and urate particles. If an acid urine is allowed to stand, it will gradually change; it becomes darker and deposits a sediment consisting of uric acid or urates, and sometimes also calcium-oxalate crystals, in which yeast-fungi and bacteria are often to be seen. This change, which the earlier investigators called "ACID FERMENTATION OF THE URINE," is generally considered as an exchange of the dihydrogen alkali phosphates with the urates of the urine. Monohydrogen phosphates besides acid urates or free uric acid or a mixture of both, according to conditions,[1] are thus formed.

Sooner or later, sometimes only after several weeks, the reaction of the original acid urine changes and becomes neutral or alkaline. The urine has now passed into the "ALKALINE FERMENTATION," which consists in the decomposition of the urea into carbon dioxide and ammonia by means of lower organisms, micrococcus ureæ, bacterium ureæ, and other bacteria. MUSCULUS[2] has isolated an enzyme from the micrococcus ureæ which decomposes urea, is soluble in water and is called *urease*. During the alkaline fermentation volatile fatty acids, especially acetic acid, may be produced, chiefly by the fermentation of the carbohydrates of the urine (SALKOWSKI[3]). A fermentation by which nitric acid is reduced to nitrous acid, and another where sulphuretted hydrogen is produced, may sometimes occur.

When the alkaline fermentation has advanced only so far as to render the reaction neutral, there often occur in the sediment fragments of uric-acid crystals, sometimes covered with prismatic crystals of alkali urate; dark-colored spheres of ammonium urate, crystals of calcium oxalate, and sometimes crystallized calcium phosphate are also found. Crystals of ammonium-magnesium phosphate (triple phosphate) and spherical ammonium urate are specially characteristic of alkaline fermentation. The urine in alkaline fermentation becomes paler and is often covered with a fine membrane which contains amorphous calcium phosphate and glistening crystals of triple phosphate and numerous micro-organisms.

NON-ORGANIZED SEDIMENTS.

Uric Acid. This acid occurs in acid urines as colored crystals which are identified partly by their form and partly by their property of giving the murexid test. On warming the urine they are not dissolved. On the addition of caustic alkali to the sediment the crystals dissolve, and when a drop of this solution is placed on a microscope-slide and treated

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[1] See Huppert-Neubauer, 10. Aufl., and A. Ritter, Zeitschr. f. Biologie, **35**.

[2] Musculus, Pflüger's Arch., **12**.

[3] Salkowski, Zeitschr. f. physiol. Chem., **13**.

with a drop of hydrochloric acid small crystals of uric acid are obtained which can be easily seen under the microscope.

Acid Urates. These occur only in the sediment of acid or neutral urines. They are amorphous, clay-yellow, brick-red, rose-colored, or brownish-red. They differ from other sediments in that they dissolve on warming the urine. They give the murexid test, and small microscopic crystals of uric acid separate on the addition of hydrochloric acid. Crystalline alkali urates occur very rarely in the urine, and as a rule only in such as have become neutral but not alkaline by alkaline fermentation. The crystals are somewhat similar to those of neutral calcium phosphate; they are not dissolved by acetic acid, however, but give a cloudiness therewith due to small crystals of uric acid.

Ammonium urate may indeed occur as a sediment in a neutral urine which at first was strongly acid and has become neutralized by the alkaline fermentation, but it is only characteristic of ammoniacal urines. This sediment consists of yellow or brownish rounded spheres which are often covered with thorny-shaped prisms and, because of this, are rather large and resemble the thornapple. It reacts to the murexid test. It is dissolved by alkalies with the development of ammonia, and crystals of uric acid separate on the addition of hydrochloric acid to this solution.

Calcium oxalate occurs in the sediment generally as small, shining, strongly refractive quadratic octahedra, which on microscopical examination remind one of a letter-envelope. The crystals can only be mistaken for small, not fully developed crystals of ammonium-magnesium phosphate. They differ from these by their insolubility in acetic acid. The oxalate may also occur as flat, oval, or nearly circular disks with central-cavities which from the side appear like an hour-glass. Calcium oxalate may occur as a sediment in an acid as well as in a neutral or alkaline urine. The quantity of calcium oxalate separated from the urine as sediment depends not only upon the amount of this salt present, but also upon the acidity of the urine. The solvent for the oxalate in the urine seems to be the diacid alkali phosphate, and the greater the quantity of this salt in the urine the greater the quantity of oxalate in solution. When, as previously mentioned (page 782), the simple-acid phosphate is formed from the diacid phosphate, on allowing the urine to stand, a corresponding part of the oxalate may be separated as sediment.

Calcium carbonate occurs in considerable quantities as sediment in the urine of herbivora. It occurs in but small quantities as a sediment in human urine, and in fact only in alkaline urines. It either has the same appearance as amorphous calcium oxalate or it occurs as somewhat larger spheres with concentric bands. It dissolves in acetic acid with the generation of gas, which differentiates it from calcium oxalate.

It is not yellow or brown like ammonium urate, and does not give the murexid test.

Calcium Phosphate. The CALCIUM TRIPHOSPHATE, $Ca_3(PO_4)_2$, which occurs only in alkaline urines, is always amorphous and occurs partly as a colorless, very fine powder and partly as a membrane consisting of very fine granules. It differs from the amorphous urates in that it is colorless, dissolves in acetic acid, but remains undissolved on warming the urine. CALCIUM DIPHOSPHATE, $CaHPO_4+2H_2O$, occurs in neutral or only in very faintly acid urine.[1] It is found sometimes as a thin film covering the urine and sometimes as a sediment. In crystallizing, the crystals may be single, or they may cross one another, or they may be arranged in groups of colorless, wedge-shaped crystals whose wide end is sharply defined. These crystals differ from crystalline alkali urates in that they dissolve without a residue in dilute acids and do not give the murexid test.

Calcium sulphate occurs very rarely as a sediment in strongly acid urine. It appears as long, thin, colorless needles, or generally as plates grouped together.

Ammonium-magnesium phosphate, TRIPLE PHOSPHATE, may separate from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine which is ammoniacal through alkaline fermentation. The crystals are so large that they may be seen with the unaided eye as colorless glistening particles in the sediment, on the walls of the vessel, and in the film on the surface of the urine. This salt forms large prismatic crystals of the rhombic system (coffin-shaped) which are easily soluble in acetic acid. Amorphous *magnesium triphosphate,* $Mg_3(PO_4)_2$, occurs with calcium triphosphate in urines rendered alkaline by a fixed alkali. Crystalline magnesium phosphate, $Mg_3(PO_4)_2+22H_2O$, has been observed in a few cases in human urine (also in horse's urine) as strongly refractive, long rhombic plates.

Kyestein is the film which appears after a little while on the surface of the urine. This coating, which was formerly considered as characteristic of urine in pregnancy, contains various elements, such as fungi, vibriones, epithelium-cells, etc. It often contains earthy phosphates and triple-phosphate crystals.

As more rare sediments we find *cystine, tyrosine, hippuric acid, xanthine, hæmatoidine.* In alkaline urine blue crytsals of *indigo* may also occur, due to a decomposition of indoxyl-glucuronic acid.

URINARY CALCULI.

Besides certain pathological constituents of the urine, all those urinary constituents which occur as sediments take part in the formation of urinary calculi. EBSTEIN[2] considers the essential difference between an

[1] C. Th. Mörner, Zeitschr. f. physiol. Chem., 58.

[2] Die Natur und Behandlung der Harnsteine. Wiesbaden, 1884.

amorphous and crystalline sediment in the urine on one side and urinary sand or large calculi on the other to be the occurrence of an organic frame in the latter. As the sediments which appear in normal acid urine and in a urine alkaline through fermentation are diverse, so also are the urinary calculi which appear under corresponding conditions.

If the formation of a calculus and its further development take place in an undecomposed urine, it is called a PRIMARY formation. If, on the contrary, the urine has undergone alkaline fermentation and the ammonia formed thereby has given rise to a calculus formation by precipitating ammonium urate, triple phosphate, and earthy phosphates, then it is called a SECONDARY formation. Such a formation takes place, for instance, when a foreign body in the bladder produces catarrh accompanied by alkaline fermentation.

We discriminate between the nucleus or nuclei—if such can be seen—and the different layers of the calculus. The nucleus may be essentially different in different cases, for quite frequently it consists of a foreign body introduced in the bladder. The calculus may have more than one nucleus. In a tabulation made by ULTZMANN of 545 cases of vesicular calculi, the nucleus in 80.9 per cent of the cases consisted of uric acid (and urates); in 5.6 per cent, of calcium oxalate; in 8.6 per cent, of earthy phosphates; in 1.4 per cent, of cystine; and in 3.5 per cent, of some foreign body.

During the growth of a calculus it often happens that, for some reason or other, the original calculus-forming substance is covered with another layer of a different substance. A new layer of the original substance may deposit on the outside of this, and this process may be repeated. In this way a calculus consisting originally of a simple stone may be converted into a so-called compound stone with several layers of different substances. Such calculi are always formed when a primary is changed into a secondary formation. By the continued action of an alkaline urine containing pus, the primary constituents of a primary calculus may be partly dissolved and be replaced by phosphates. Metamorphosed urinary calculi are formed in this way.

Uric-acid calculi are very abundant. They are variable in size and form. The size of the bladder-stone varies from that of a pea or bean to that of a goose-egg. Uric acid stones are always colored; generally they are grayish-yellow, yellowish-brown, or pale red-brown. The upper surface is sometimes entirely even or smooth, sometimes rough or uneven. Next to the oxalate calculus the uric-acid calculus is the hardest. The fractured surface shows regular concentric, unequally colored layers which may often be removed as shells. These calculi are formed primarily. Layers of uric acid sometimes alternate with other layers of primary formation, most frequently with layers of calcium oxalate.

The simple uric-acid calculus leaves very little residue when burnt on a platinum foil. It gives the murexid test, but there is no material development of ammonia when acted on by caustic soda.

Ammonium urate calculi occur as primary calculi in new-born or nursing infants, rarely in grown persons. They often occur as a secondary formation. The primary stones are small, with a pale-yellow or dark-yellowish surface. When moist they are almost like dough; in the dry state they are earthy, easily crumbling into a pale powder. They give the murexid test and develop much ammonia with caustic soda.

Calcium-oxalate calculi are, next to uric-acid calculi, the most abundant. They are either smooth and small (HEMP-SEED CALCULI) or larger, of the size of a hen's egg, with rough, uneven surface, or their surface is covered with prongs (MULBERRY CALCULI). These calculi produce bleeding easily, and therefore they often have a dark-brown surface due to decomposed blood-coloring matters. Among the calculi occurring in man these are the hardest. They dissolve in hydrochloric acid without developing gas, but are not soluble in acetic acid. After gently heating the powder, it dissolves in acetic acid with frothing. With more intense heat it becomes alkaline, due to the production of quicklime.

Phosphate Calculi. These, which consist mainly of a mixture of the normal phosphate of the alkaline earths with triple phosphate, may be very large. They are as a rule of secondary formation and contain besides these phosphates also some ammonium urate and calcium oxalate. These calculi ordinarily consist of a mixture of three constituents—earthy phosphate, triple phosphate, and ammonium urate—surrounding a foreign body as a nucleus. Their color is variable—white, dingy white, pale yellow, sometimes violet or lilac-colored (from indigo red). The surface is always rough. Calculi consisting of triple phosphate alone are seldom found. They are ordinarily small, with granular or radiated crystalline fracture. Stones of mono-acid calcium phosphate are also seldom obtained. They are white and have beautiful crystalline texture. The phosphatic calculi do not burn up, the powder dissolves in acid without effervescence, and the solution gives the reactions for phosphoric acid and the alkaline earths. The triple-phosphate calculi generate ammonia on the addition of an alkali.

Calcium-carbonate calculi occur chiefly in herbivora. They are seldom found in man. They have mostly chalky properties, and are ordinarily white. They are completely or in great part dissolved by acids with effervescence.

Cystine calculi occur but seldom. They are of primary formation, of various sizes, sometimes as large as a hen's egg. They have a smooth or rough surface, are white or pale yellow, and have a crystalline fracture. They are not very hard and are consumed almost entirely on the platinum foil burning with a bluish flame. They give the above-mentioned reactions for cystine.

Xanthine calculi are very rarely found. They are also of primary formation. They vary from the size of a pea to that of a hen's egg. They are whitish, yel-

lowish-brown or cinnamon-brown in color, of medium hardness, with amorphous fracture, and on rubbing appear like wax. They burn up completely when heated on a platinum foil. They give the xanthine reaction with nitric acid and alkali, but this must not be mistaken for the murexid test.

Urostealith calculi have been observed only a few times. In the moist state they are soft and elastic at the temperature of the body, but in the dry state they are brittle, with an amorphous fracture and waxy appearance. They burn with a luminous flame when heated on platinum foil and generate an odor similar to resin or shellac. Such a calculus, investigated by KRUKENBERG,[1] consisted of paraffin derived from a paraffin bougie used as a sound on the patient. Perhaps the urostealith calculi observed in other cases had a similar origin, although the substances of which they consisted have not been closely studied. HORBACZEWSKI has recently analyzed a case of urostealith which, to all appearances, was formed in the bladder. This calculus contained 25 p. m. water, 8 p. m. inorganic bodies, 117 p. m. bodies insoluble in ether, and 850 p. m. organic bodies soluble in ether, among which were 515 p. m. free fatty acids, 335 p. m. fat, and traces of cholesterin. The fatty acids consisted of a mixture of stearic, palmitic, and probably myristic acids.

HORBACZEWSKI [2] has also analyzed a bladder stone which contained 958.7 p. m. *cholesterin*.

Fibrin calculi sometimes occur. They consist of more or less changed fibrin-coagulum. On burning they develop an odor of burnt horn.

The *chemical investigation of urinary calculi* is of great practical importance. To make such an examination actually instructive it is necessary to investigate, separately, the different layers which constitute the calculus. For this purpose saw the calculus, previously wrapped in paper, with a fine saw so that the nucleus becomes accessible. Then peel off the different layers, or, if the stone is to be kept, scrape off enough of the powder from each layer for examination. This powder is then tested by heating on the platinum foil. It must not be forgotten that a calculus is never entirely burnt up, and also that it is never so free from organic matter that on heating it does not carbonize. Do not, therefore, lay too great stress on a very insignificant unburnt residue or on a very small amount of organic matter, but consider the calculus in the former case as completely burnt and in the latter as unaffected.

When the powder is in great part burnt up, but a significant quantity of unburnt residue remains, then the powder in question contains as a rule urates mixed with inorganic bodies. In such cases remove the urate with boiling water and then test the filtrate for uric acid and the suspected bases. The residue is then tested according to the following *scheme* of HELLER, which is well adapted to the investigation of urinary calculi. In regard to the more detailed examination the reader is referred to special works on the subject.

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[1] Chem. Untersuch. z. wissensch. Med., **2**. Cited from Maly's Jahresber., **19**, 422.

[2] Zeitschr. f. physiol. Chem., **18**.

On heating the powder on platinum foil, it		
Does burn		
Without flame		
The powder gives the murexid test		
No noticeable ammonia reaction		Uric acid
Strong ammonia reaction		Ammonium urate
Does not give the murexid test. The powder dissolves in HNO_3 without effervescence. The dried yellow residue becomes orange with alkali, beautiful red on warming		Xanthine
With flame		
Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia		Cystine
Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether		Urostealith
Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in KHO with heat. Precipitated herefrom by acetic acid and generation of H_2S		Fibrin
Does not burn		
The powder when treated with HCl		
Effervesces		Calcium carbonate
Does not effervesce		
The powder gently heated and treated with HCl		
Effervesces		Calcium oxalate
The powder when moistened with a little KHO		
No NH_3, or at least only traces of NH_3. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous)		Bone earth (phosphate of calcium and magnesium)
Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives a crystalline precipitate with ammonia		Triple phosphate (mixed with unknown amount of earthy phosphate)

CHAPTER XVI.

THE SKIN AND ITS SECRETIONS.

In the structure of the skin of man and vertebrates many different kinds of substances occur which have already been considered, such as the constituents of the epidermal formation, the connective and fatty tissues, the nerves, muscles, etc. Among these the different horn structures, the hair, nails, etc., whose chief constituent, keratin, has been spoken of in another chapter (Chapter II), are of special interest.

The cells of the horny structure show, in proportion to their age, a different resistance to chemical reagents, especially fixed alkalies. The younger the horn-cell the less resistance it has to the action of alkalies; with advancing age the resistance becomes greater, and the cell-membranes of many horn-formations are nearly insoluble in caustic alkalies. Keratin (or the keratins) occurs in the horn structure mixed with other bodies, from which it is isolated with difficulty. These are detected by microchemical investigations, and according to Unna[1] three different substances can be detected in the horn substance, designated by him *A*, *B* and *C* keratin.

The *A*-keratin, which forms the envelope of the horn and hair cells and the outer layer of the hair, is the purest keratin. It is not dissolved by fuming nitric acid at the ordinary temperature and does not give the xanthoproteic reaction, and its keratin nature is doubtful. The *B*-keratin, which occurs as the contents of the nail cells, gives the xanthoproteic reaction like the *C*-keratin occurring in hair, but differs from the *C*-keratin by being soluble in fuming nitric acid.

Besides these substances, which have been called keratins, the horn structure also contains other proteins which are soluble in pepsin-hydrochloric acid. Among these we find residue of nuclei and the so-called trichohyalin in the hair, which is a substance of unknown constitution and characterized by great insolubility. From these statements it is evident that we are here dealing with a mixture of different substances and for this reason it is unnecessary to give the older elementary analyses of the various epidermoidal structures.

The quantity of sulphur and of mineral bodies is of certain interest. The sulphur and cystine content of these structures can be found on

[1] Monatsch. f. prakt. Dermat., 44.

pages 112, 113 and in this connection it must be mentioned that, according to the investigations of RUTHERFORD and HAWK,[1] the sulphur content of human hair is higher in men than in women, at least for the Caucasian race, and also that red hair has the highest sulphur content irrespective of race or gender. Hair on incineration leaves considerable ash, which in human hair varies between 2.6 and 16 p. m., and in animal hair is still greater, even up to 71 p. m. in the hair of the deer. The ash consists of large amounts of alkali and calcium sulphate, and its sulphur probably originates from the organic substance, which make the statements as to the composition of the ash of hair of little value. Calcium occurs in larger amounts, especially phosphate as well as carbonate, and is most abundant in white hair. The amount of iron oxide in 1000 grams of the ash of human hair varies between 42.2 grams in blond and 108.7 grams in brown hair, and silicic acid between 66.1 grams in black and 424.6 grams in red hair (BAUDRIMONT). The nails are rich in calcium phosphate and the feathers rich in silicic acid, especially the feathers of grain-eating birds. According to v. GORUP-BESANEZ[2] the quantity of silicic acid in grain-eating birds was 400 p. m., and in meat, berries and insect-eating birds the amount was only 270 p. m. of the total ash. DRECHSEL[3] claims that at least a part of the silicic acid exists in the feathers in organic combination as an ester.

According to GAUTIER and BERTRAND[4] arsenic also occurs in the epidermal formations. GAUTIER says that arsenic is of importance in the formation and growth of the formations, and on the other hand the hair, nails, and epidermis-cells, are of great importance in the excretion of arsenic.

The skin of invertebrates has been the subject, in a few cases, of chemical investigation, and in these animals various substances have been found, of which a few, though little studied, are worth discussing. Among them *tunicin*, which is found especially in the mantle of the tunicata, and the widely diffused *chitin*, found in the cuticle-formation of invertebrates, are of interest.

Tunicin. Cellulose seems, from the investigations of AMBRONN, to occur rather extensively in the animal kingdom in the arthropoda and the mollusks. It has been known for a long time as the mantle of the *tunicata*, and this animal cellulose was called tunicin by BERTHELOT. According to the investigations of WINTERSTEIN there does not seem to exist any marked difference between tunicin and ordinary vegetable cellulose. On boiling with dilute acid, tunicin yields dextrose, as shown first by FRANCHIMONT[5] and later confirmed by WINTERSTEIN.

[1] Journ. of Biol. Chem., **3**.

[2] Lehr. d. physiol. Chem., 4. Aufl., 660, 661; Baudrimont, *ibid.*

[3] Centralbl. f. Physiol., **11**, 361.

[4] Gautier, Compt. rend., **129**, **130**, **131**; Bertrand, *ibid.*, **134**.

[5] Ambronn, Maly's Jahresber., **20**; Berthelot, Annal. de Chim. et Phys., **56**, Compt.

Chitin is not found in vertebrates. In invertebrates chitin is alleged to occur in several classes of animals; but it can be positively asserted that true, typical chitin is found only in cephalopods and in articulated animals especially, in which it forms the chief organic constituent of the shell, etc. KRAWKOW [1] found that chitin of the shell, etc., does not seem to occur free, but in combination with another substance, probably a protein-like body. In GILSON and WINTERSTEIN'S [2] investigations chitin occurred in certain fungi as well.

According to SUNDVIK the formula of chitin is probably $C_{60}H_{100}N_8O_{38} + n(H_2O)$, where n may vary between 1 and 4. ARAKI claims that it has on the contrary the composition $C_{18}H_{30}N_2O_{12}$. KRAWKOW found that the chitins of different origin show different action with iodine, and he therefore concludes that there must exist quite a group of chitins, which seem to be amino derivatives of different carbohydrates, such as dextrose, glycogen, dextrins, etc. According to ZANDER [3] only two chitins exist, one of which turns violet with iodine and zinc chloride, and the other brown.

Chitin is decomposed on boiling with mineral acids and yields, as shown by LEDDERHOSE, *glucosamine* and *acetic acid*. HOPPE-SEYLER and ARAKI found on heating with alkali and a little water to 180° that chitin was split into a new substance, *chitosan*, and acetic acid, and that this chitosan contained acetyl groups as well as glucosamine. Chitin can hardly be considered as a simple acetyl glucosamine, but probably has a much more complicated constitution, which is not known at the present. FRÄNKEL and KELLY as well as OFFER [4] have obtained acetyl glucosamine, $C_6H_{11}O_5NH(COCH_3)$ and acetyldiglucosamine $(C_{12}H_{23}N_2O_9)$ $COCH_3$ as characteristic cleavage products.

The acetyldiglucosamine has the same empirical formula, $C_{14}H_{26}N_2O_{10}$, as the chitosan prepared by ARAKI, but it is not identical therewith. The chitosan, which v. FÜRTH and RUSSO [5] obtained as a crystalline hydrochloric acid combination, has a different composition, and according to them, is more likely a multiple of $C_{13}H_{26}N_2O_{14}$. On heating with acetic anhydride chitosan is converted into a chitin-like substance which is not identical with chitin. Chitosan is insoluble in water and alkali, but dissolves in dilute acids. It splits into acetic acid and glucosamine by the action of hydrochloric acid. According to v. FÜRTH and

rend., **47**; Winterstein, Zeitschr. f. physiol. Chem., **18**; Franchimont, Ber. d. deutsch chem. Gesellsch., **12**.

[1] Zeitschr. f. Biologie, **29**.

[2] Gilson, Compt. rend., **120**; Winterstein, Ber. d. deutsch. chem. Gesellsch., **27** and **28**.

[3] Sundvik, Zeitschr. f. physiol. Chem., **5**; Araki, *ibid.*, **20**; Zander, Pflüger's Arch., **66**.

[4] Ledderhose, Zeitschr. f. physiol. Chem., **2** and **4**; Araki, l. c.; Fränkel and Kelly, Monatshefte f. Chem., **23**; Offer, Bioch. Zeitschr., **7**.

[5] Hofmeister's Beiträge, **8**.

Russo on acid cleavage it yields 25 per cent acetic acid and 60 per cent glucosamine.

In a dry state chitin forms a white, brittle mass retaining the form of the original tissue. It is insoluble in boiling water, alcohol, ether, acetic acid, dilute mineral acids, and dilute alkalies. It is soluble in concentrated acids. It is dissolved without decomposing in cold concentrated hydrochloric acid, but is decomposed by boiling hydrochloric acid. According to Krawkow the various chitins behave differently with iodine or with sulphuric acid and iodine, in that some are colored reddish brown, blue, or violet, while others are not colored at all.

Chitin may be easily prepared from the wings of insects or from the shells of the lobster or the crab, the last-mentioned having first been extracted by an acid so as to remove the lime salts. The wings or shells are boiled with caustic alkali until they are white, afterward washed with water, then with dilute acid and water. The pigments remaining can be destroyed by permanganate. The excess of this last can be removed by a dilute solution of bisulphite, washed with water and then extracted with alcohol and ether.

Hyalin is the chief organic constituent of the walls of hydatid cysts. From a chemical point of view it stands close to chitin, or between it and protein. In old and more transparent sacs it is tolerably free from mineral bodies, but in younger sacs it contains a great quantity (16 per cent) of lime salts (carbonate, phosphate, and sulphate).

According to Lücke [1] its composition is:

	C	H	N	O
From old cysts	45.3	6.5	5.2	43.0
From young cysts	44.1	6.7	4.5	44.7

It differs from keratin on the one hand and from proteins on the other by the absence of sulphur, also by its yielding, when boiled with dilute sulphuric acid, a variety of sugar in large quantities (50 per cent), which is reducing, fermentable, and dextrogyrate. It differs from chitin by the property of being gradually dissolved by caustic potash or soda, or by dilute acids; also by its solubility on heating with water to 150° C.

The coloring matters of the skin and horn-formations are of different kinds, but have not been extensively studied. Those occurring in the stratum Malpighii of the skin, especially of the negro, and the black or brown pigment occurring in the hair, belong to the group of those substances which have received the name *melanins*.

Melanins. This group includes several different varieties of amorphous black or brown pigments which are insoluble in water, alcohol, ether, chloroform, and dilute acids, and which occur in the skin, hair, epithelium-cells of the retina, in sepia, in certain pathological formations, and in the blood and urine in disease. Of these pigments there are a few, such as

[1] Virchow's Arch., 19.

SCHMIEDEBERG'S *sarcomelanin*, and that from the melanotic sarcomata of horses, the *hippomelanin* (NENCKI, SIEBER, and BERDEZ), which are soluble with difficulty in alkalies, while others, such as the coloring matter of certain pathological swellings in man, the *phymatorhusin* (NENCKI and BERDEZ), are readily soluble in alkalies. The humus-like products, called *melanoidic acids* by SCHMIEDEBERG, obtained on boiling proteins with mineral acids, are rather easily soluble in alkalies.

Among the melanins there are a few, for example the choroid pigment, which are free from sulphur (LANDOLT and others); others, on the contrary, as sarcomelanin and the pigment of the hair and of horse-hair, are rather rich in sulphur (2–4 per cent), while the phymatorhusin found in certain swellings and in the urine (NENCKI and BERDEZ, K. MÖRNER) is very rich in sulphur (8–10 per cent). Whether any of these pigments, especially the phymatorhusin, contains any iron or not is an important though disputed point, for it leads to the question whether these pigments are formed from the blood-coloring matters. According to NENCKI and BERDEZ the pigment, phymatorhusin, isolated by them from a melanotic sarcoma did not contain any iron, and according to them is not a derivative of hæmoglobin. K. MÖRNER and later also BRANDL and L. PFEIFFER found, on the contrary, that this pigment did contain iron, and they consider it as a derivative of the blood-pigments. The *sarcomelanin* (from a sarcomatous liver) analyzed by SCHMIEDEBERG contained 2.7 per cent iron, which was partly in organic combination and could not be completely removed by dilute hydrochloric acid. The *sarcomelanic acid* prepared by SCHMIEDEBERG by the action of alkali on this melanin contained 1.07 per cent iron. The sarcomelanin investigated by ZDAREK and v. ZEYNEK also contained 0.4 per cent iron. Recently WOLFF[1] prepared two pigments from a melanotic liver, of which one was no doubt modified. The other, which was soluble in a soda solution, contained 2.51 per cent sulphur and 2.63 per cent iron, which was in great part split off by 20-per cent hydrochloric acid. From another liver he, on the contrary, obtained melanin free from iron but with 1.67 per cent sulphur. From this melanin he obtained, by treatment with bromine, a hydro-aromatic body which was related to xyliton (a condensation product of acetone). A similar product could not be obtained from the pigment of the hair (SPIEGLER) nor from hippomelanin (v. FÜRTH and JERUSALEM[2]).

[1] Zdarek and v. Zeynek, Zeitschr. f. physiol. Chem., **36**; Wolff, Hofmeister's Beiträge, **5**. The literature on the melanins may be found in Schmiedeberg, "Elementarformeln einiger Eiweisskörper, etc." Arch. f. exp. Path. u. Pharm., **39**; also in Kobert, Wiener Klinik, **27** (1901), and Spiegler, Hofmeister's Beiträge, **4**, and especially v. Fürth, Centralbl. f. allg. Path. u. Path. Anat., **15**, 1907, 617.

[2] Wolff, Hofmeister's Beiträge, **5**; Spiegler, *ibid.*, **10**; v. Fürth and Jerusalem, *ibid.*, 10.

The difficulties which attend the isolation and purification of the melanins have not been overcome in certain cases, while in others it is questionable whether the final product obtained has not another composition from the original coloring matter, owing to the energetic chemical processes resorted to in its purification. The elementary composition shows widely varying results in the different melanins, namely, 48–60 per cent carbon and 8–14 per cent nitrogen. Under these circumstances and as no doubt we have a large number of melanins having different composition, it seems that a tabulation of the analyses of the different preparations can be of secondary importance only.

So little is known about the structural products of the melanins or melanoids that it is impossible to give the origin of these bodies. As undoubtedly there are several distinct melanins, their origin must also be distinct. The ferruginous melanins should be considered as originating from the blood-pigments until further research proves otherwise. Others, on the contrary, cannot have this origin; for example, the pigments of the hair and choroid, which are free from iron and which do not yield hæmopyrrol according to SPIEGLER. Several melanins—and this is also true of the melanoids produced from proteins on cleavage with acids (SAMUELY [1])—yield indol or skatol and a pyrrol substance on fusion with alkali, while hippomelanin, according to v. FÜRTH and JERUSALEM, gives a fecal odor on this treatment, but does not yield any indol or skatol. More characteristic than the last two mentioned bodies is a phenol-like substance, which occurs to a slight extent, and gives a bluish-black color with ferric chloride (v. FÜRTH).

The cyclic complexes of the proteins are rightly considered as the mother-substance of the melanins (SAMUELY and v. FÜRTH and others), and this view has received support by the behavior of tyrosine with oxidases. It has been found that by the action of a plant oxidase, BERTRAND'S tyrosinase [2], upon tyrosine, colored products and then melanin-like substances are formed. v. FÜRTH with SCHNEIDER and PRIBRAM, GESSARD, NEUBERG, DEWITZ and others [3] have shown that similar acting tyrosinases also occur in the animal kingdom, in insects and sepia, in melanotic tumors and in pigmented skin, and v. FÜRTH and JERUSALEM have prepared an artificial melanin from tyrosine which shows great similarity to hippomelanin. Finally NEUBERG [4] has also prepared an extract from the melanotic metastases of a primary adrenal tumor which formed a dark-brown pigment from adrenalin and *p*-oxyphenylethylamine, but not from tyrosine. As indicated

[1] Hofmeister's Beiträge, **2**.

[2] Compt. rend., **122**.

[3] The literature can be found in v. Fürth and Jerusalem, Hofmeister's Beiträge, **10**.

[4] Virchow's Arch., **192**.

above, we tend more and more to accept the view that the melanins are derived from the cyclic components of the proteins.

In addition to the coloring matters of the human skin it is in place here to treat of the pigments found in the skin or epidermal formation of animals.

The beautiful color of the feathers of many birds depends in certain cases on purely physical causes (interference-phenomena), but in other cases on coloring matters of various kinds. Such a coloring matter is the amorphous reddish-violet *turacin*, which contains 7 per cent copper and whose spectrum is very similar to that of oxyhæmoglobin. It must be remarked that according to LAIDLAW [1] turacin or at least a pigment with the same properties can be obtained on boiling hæmatoporphyrin in dilute ammonia with ammoniacal copper solution. KRUKENBERG [2] found a large number of coloring matters in birds' feathers, namely, *zooerythrin, zoofulvin, turacoverdin, zoorubin, psittacofulvin*, and others which cannot be enumerated here.

Tetronerythrin, so named by WURM, is a red amorphous pigment which is soluble in alcohol and ether, and which occurs in the red warty spots over the eyes of the heathcock and the grouse, and which is very widely spread among the invertebrates (HALLIBURTON, DE MEREJKOWSKI, MACMUNN). Besides tetronerythrin MACMUNN found in the shells of crabs and lobsters a blue coloring matter *cyanocrystallin*, which turns red with acids and by boiling water. *Hæmatoporphyrin*, according to MACMUNN,[3] also occurs in the integuments of certain of the lower animals.

In certain butterflies (the pieridinæ) the white pigment of the wings consists, as shown by HOPKINS,[4] of uric acid, and the yellow pigment of a uric-acid derivative, *lepidotic acid*, which yields a purple substance, *lepidoporphyrin*, on warming with dilute sulphuric acid. The yellow and red pigment of the Vanessa are, according to LINDEN,[5] of an entirely different kind. In this case we are dealing with a compound between protein and a pigment which is allied to bilirubin or urobilin, i.e., a compound similar to hæmoglobin.

In addition to the coloring matters thus far mentioned a few others found in certain animals (though not in the skin) will be spoken of.

Carminic acid, or the red pigment of the cochineal, gives on oxidation, according to LIEBERMANN and VOSWINCKEL,[6] *cochenillic acid*, $C_{10}H_8O_7$, and *coccinic acid*. $C_9H_8O_5$, the first being the tri-carboxylic acid, and the other the di-carboxylic acid, of *m*-cresol. The beautiful purple solution of ammonium carminate has two absorption-bands between *D* and *E* which are similar to those of oxyhæmoglobin. These bands lie nearer to *E* and closer together and are less sharply defined. *Purple* is the evaporated residue from the purple-violet secretion, caused by the action of the sunlight, upon the so-called " purple gland " of the mantle of certain species of *murex* and *purpura*. Its chemical nature has not been investigated.

Among the remaining coloring matters found in invertebrates may be mentioned *blue stentorin, actiniochrom, bonellin, polyperythrin, pentacrinin, antedonin, crustaceorubin, janthinin*, and *chlorophyll*.

Sebum when freshly secreted is an oily semi-fluid mass which solidifies on the upper surface of the skin, forming a greasy coating. RÖHMANN

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[1] Journ. of Physiol., **31**.

[2] Vergleichende physiol. Studien, Abth. **5**, and (2. Reihe) Abth. **1**, 151, Abth. **2**, 1, and Abth. **3**, 128.

[3] Wurm, cited from Maly's Jahresber., **1**; Halliburton, Journ. of Physiol., **6**; Merejkowski, Compt. rend., **93**; MacMunn, Proc. Roy. Soc., 1883, and Journ. of Phyisol., **7**.

[4] Phil. Trans., **186**.

[5] Pflüger's Arch., **98**.

[6] Ber. d. deutsch. chem. Gesellsch., **30**.

and LINSER hold that sebum is a mixture of the secretion of the sebaceous glands and of the constituents of the epidermis. HOPPE-SEYLER found, in the sebum, a body similar to casein besides albumin and fat, while RÖHMANN and LINSER claim that true fat occurs only to a very slight extent. On saponification the sebum gives an oil, *dermolein*, which combines readily with iodine, and another body, *dermocerin*, which melts at 64–65° and which occurs to a considerable extent in dermoid cysts, and which is perhaps identical with the constituent of cysts called cetyl alcohol by v. ZEYNEK. According to AMESEDER this dermocerin is not a pure substance, and the cetyl alcohol obtained from the fat of dermoid cysts is an *eicosyl alcohol*, $C_{20}H_{42}O$, corresponding to arachinic acid. Cholesterin is found in especially large quantities in the *vernix caseosa*. RÜPPEL[1] found on an average in the *vernix caseosa* 348.52 p. m. water and 138.72 p. m. ether extractives and also mentions the presence of isocholesterin. These claims are disputed by UNNA.[2] In his experience isocholesterin does not occur in the vernix fat nor in the sebum of man, although all kinds of sebum contain cholesterin.

On account of the opinion generally held that the wax of the plant epidermis serves as protection for the inner parts of the fruit and plant, LIEBREICH[3] has suggested that these combinations of fatty acids with monatomic alcohols are the cause of the waxes having a greater resistance as compared with the glycerin fats. He also considers that the cholesterin fats play the rôle of a protective fat in the animal kingdom, and he has been able to detect cholesterin fat in human skin and hair, in *vernix caseosa*, whalebone, tortoise-shell, cow's horn, the feathers and beaks of several birds, the spines of the hedgehog and porcupine, the hoofs of horses, etc. He draws the following conclusion from this, namely, that the cholesterin fats always appear in combination with the keratinous substance, and that the cholesterin fat, like the wax of plants, serves as protection for the skin-surface of animals. Of the sebum fats investigated by UNNA all contained, with the exception of the epidermis fat, besides cholesterin, greater or smaller amounts of cholesterin ester. The epidermis fat, on the contrary, was almost free from esters and consisted chiefly of free cholesterin.

In the fatty protective substance secreted by the Psylla alni SUNDVIK[4] found psylla-alcohol, $C_{33}H_{68}O$, which exists there as an ester in combination with psyllic acid, $C_{32}H_{65}COOH$. This alcohol has also been found in the wax of the humble-bee.

[1] Hoppe-Seyler, Physiol. Chem., 760; Linser with Röhmann, Centralbl. f. Physiol., **19**, 317; see also reference in *ibid.*, 18, from Deutsch. Arch. f. klin. Med., 1904; Rüppel, Zeitschr. f. physiol. Chem., **21**; Ameseder, *ibid.*, 52.

[2] Monatsch. f. prakt. Dermat., **45**.

[3] Virchow's Arch., **121**.

[4] Zeitschr. f. physiol. Chem., **17**, **25**, **32**, **53** and **54**.

Cerumen is a mixture of the secretion of the sebaceous and sweat glands of the cartilaginous part of the outer passages of the ear. It chiefly contains soaps and fat, fatty acids, cholesterin and protein, and besides these a red substance easily soluble in alcohol and with a bitter-sweet taste.[1]

The **preputial secretion,** *smegma præputii*, contains chiefly fat, also cholesterin and ammonium soaps, which probably are produced from decomposed urine. The hippuric acid, benzoic acid, and calcium oxalate found in the smegma of the horse probably have the same origin.

We may also consider as a preputial secretion the *castoreum*, which is secreted by two peculiar glandular sacs in the prepuce of the beaver. The castoreum is a mixture of proteins, fats, resins, traces of phenol (volatile oil), and a non-nitrogenous body, *castorin*, crystallizing from alcohol in four-sided needles, insoluble in cold water, but somewhat soluble in boiling water, and whose composition is little known.

In the secretion from the anal glands of the skunk, butyl mercaptan and alkyl sulphides have been found (Aldrich, E. Beckmann [2]).

Wool-fat, or the so-called fat-sweat of sheep, is a mixture of the secretion of the sudoriparous and sebaceous glands. There is found in the watery extract a large quantity of potassium which is combined with organic acid, volatile and non-volatile fatty acids, benzoic acid, phenol-sulphuric acid, lactic acid, malic acid, succinic acid, and others. The fat contains, among other bodies, abundant quantities of ethers of fatty acids with cholesterin and isocholesterin. Darmstädter and Lifschütz have found other alcohols in wool-fat besides myristic acid, also two oxyfatty acids, *lanoceric acid*, $C_{30}H_{60}O_4$, and *lanopalmitic acid*, $C_{16}H_{32}O_3$. Isocholesterin, oxycholesterin and carnaubyl alcohol, $C_{24}H_{49}OH$, are besides the two last-mentioned acids, substances that are characteristic of wool-fat. According to Röhmann [3] wool-fat contains a body *lanocerin*, which is the internal anhydride of the above-mentioned lanoceric acid.

The secretion of the coccygeal glands of ducks and geese contains a body similar to casein, besides albumin, nuclein, lecithin, and fat, but no sugar (De Jonge). The chief constituent is *octadecyl alcohol*, $C_{18}H_{38}O$, which represents 40–45 per cent of the ethereal extract (Röhmann). The fatty acids are oleic acid, small amounts of caprylic acid, palmitic acid, and stearic acid, and optical isomers of lauric and myristic acid. The fatty acids are in great part combined with the octadecylic acid, and this is probably formed by the reduction of stearic acid or oleic acid. The secretion also contains a substance related to lanocerin which Röhmann calls *pennacerin*. Poisonous bodies have been found in the secretion of the skin of the salamander and the toad, namely, *samandarin* (Zaleski, Faust) and *bufidin* (Jornara and Casali), *bufotalin* and the disputed bodies *bufonin* and *bufotenin* (Faust, Bertrand and Phisalix [4]). *Thalassin* is the crystalline body discovered by Richet [5] which is the poisonous constituent of the feelers of the sea nettle.

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[1] See Lamois and Martz, Maly's Jahresber., **27**, 40.

[2] Aldrich, Journ. of Exp. Med., **1**; Beckmann, Maly's Jahresber., **26**, 566.

[3] Darmstädter and Lifschütz, Ber. d. d. Chem., Gesellsch., **29** and **31**; Röhmann, Hofmeister's Beiträge, **5**, and Centralbl. f. Physiol., **19**, 317. See also Unna, l. c., **45**, and Lifschütz and Unna, *ibid.*, 234.

[4] De Jonge, Zeitschr. f. physiol. Chem., **3**; Röhmann l. c.; Zaleski, Hoppe-Seyler's Med.-chem. Untersuch., p. 85; Faust, Arch. f. exp. Path. u. Pharm., **41**; Jornara and Casali, Maly's Jahresbr., **3**; Faust, Arch. f. exp. Path. u. Pharm., **47** and **49**; Bertrand, Compt. rend., **135**; Bertrand and Phisalix, *ibid.*

[5] Pflüger's Arch., **108**.

The Perspiration. Of the secretions of the skin, whose quantity amounts to about $\frac{1}{64}$ of the weight of the body, a disproportionately large part consists of water. Next to the kidneys, the skin, in man, is the most important means for the elimination of water. As the glands of the skin and the kidneys stand near to each other in regard to their functions, they may to a certain extent act vicariously.

The circumstances which influence the secretion of perspiration are numerous, and the quantity of sweat secreted must consequently vary considerably. The secretion differs in different parts of the skin, and it has been stated that the perspiration of the cheek, that of the palm of the hand, and that under the arm stand to each other as 100:90:45. From the unequal secretion on different parts of the body it follows that no results as to the quantity of secretion for the entire surface of the body can be calculated from the quantity secreted by a small part of the skin in a given time. In determining the total quantity a stronger secretion is as a rule produced, and as the glands can with difficulty work for a long time with the same energy, it is hardly correct to estimate the quantity of secretion per day from a strong secretion during only a short time.

The perspiration obtained for investigation is never quite pure, but contains cast-off epidermis-cells, also cells and fat-globules from the sebaceous glands. Filtered perspiration is a clear, colorless fluid with a salty taste and of different odors from different parts of the body. The physiological reaction is acid, according to most reports. Under certain conditions an alkaline sweat may be secreted (Trümpy and Luchsinger, Heuss). An alkaline reaction may also depend on a decomposition with the formation of ammonia. According to a few investigators the physiological reaction is alkaline, and an acid reaction depends, upon an admixture of fatty acids from the sebum. Camerer found that the reaction of human perspiration in certain cases was acid and in others alkaline. Moriggia found that the sweat from herbivora was ordinarily alkaline, while that from carnivora was generally acid. Smith [1] showed that horse's sweat is strongly alkaline.

The specific gravity of human perspiration varies between 1.001 and 1.010. It contains 977.4–995.6 p. m., average about 982 p. m. water. The solids are 4.4–22.6 p. m. The molecular concentration also varies widely and the freezing-point depression depends essentially upon the content of NaCl. Ardin-Delteil found $\Delta = -0.08$–$0.46°$, average–0.237°. Brieger and Disselhorst found with perspiration

[1] Trümpy and Luchsinger, Pflüger's Arch., **18**; Heuss, Maly's Jahresber., **22**; Camerer, Zeitschr. f. Biologie, **41**; Moriggia, Moleschott's Untersuch. zur Naturlehre, **11**; Smith, Journ. of Physiol., **11**. In regard to the older literature on perspiration, see Hermann's Handbuch, **5**, Thl. 1, 421 and 543.

containing 2.9, 7.07 and 13.5 p. m. NaCl, that the Δ was equal to $-0.322°$, $-0.608°$ and $-1.002°$, respectively. TARUGI and TOMASINELLI [1] found Δ to be 0.52° as an average. The organic bodies are *neutral fats, cholesterin, volatile fatty acids*, traces of *protein* (according to LECLERC and SMITH always in horses, and according to GAUBE regularly in man, while LEUBE [2] claims only occasionally after hot baths, in BRIGHT'S disease, and after the use of pilocarpin), *creatinine* (CAPRANICA), *aromatic oxyacids, ethereal-sulphuric acids* of *phenol* and *skatoxyl* (KAST [3]), sometimes also of *indoxyl*, and lastly *urea*. The quantity of urea has been determined by ARGUTINSKY. In two steam-bath experiments, in which in the course of $\frac{1}{2}$ and $\frac{3}{4}$ hour respectively he obtained 225 and 330 cc. of perspiration, he found 1.61 and 1.24 p. m. urea. Of the total nitrogen of the perspiration in these two experiments 68.5 per cent and 74.9 per cent respectively belong to the urea. From ARGUNTINSKY'S experiments, and also from those of CRAMER,[4] it follows that of the total nitrogen a portion, not to be disregarded, is eliminated by the perspiration. This portion was indeed 12 per cent, in an experiment of CRAMER, at high temperature and powerful muscular activity, and ZUNTZ and his collaborators find indeed more than 13 per cent in high altitudes. CRAMER also found ammonia in the perspiration. In uræmia and in anuria in cholera, urea may be secreted in such quantities, by the sweat-glands, that crystals deposit upon the skin. The mineral bodies consist chiefly of sodium chloride with some potassium chloride, alkali sulphate, and phosphate. The relative quantities of these in perspiration differ materially from the amount in the urine (FAVRE,[5] KAST). The relation, according to KAST, is as follows:

	Chlorine	:	Phosphate	:	Sulphate
In perspiration	1	:	0.0015	:	0.009
In urine	1	:	0.1320	:	0.397

KAST found that the proportion of ethereal-sulphuric acid to the sulphate-sulphuric acid in perspiration was 1:12. After the administration of aromatic substances the ethereal-sulphuric acid does not increase to the same extent in the perspiration as in the urine (see Chapter XV). The quantity of mineral substances was on an average 7 p. m.

Sugar may pass into the perspiration in diabetes, but the passage of the bile-coloring matters has not been positively shown in this secretion. *Benzoic acid, succinic acid, tartaric acid, iodine, arsenic, mercuric chloride*, and *quinine* pass

[1] Ardin-Delteil, Maly's Jahresber., **30**; Brieger and Disselhorst, Deutsch. med. Wochenschr., **29**; Tarugi and Tomasinelli, cited in Physiol. Centralbl., **22**, 748.

[2] Leclerc, Compt. rend., **107**; Gaube, Maly's Jahresber., **22**; Leube, Virchow's Arch., **48** and **50**, and Arch. f. klin. Med., **7**.

[3] Capranica, Maly's Jahresber., **12**; Kast, Zeitschr. f. physiol. Chem., **11**.

[4] Argutinsky, Pflüger's Arch., **46**; Cramer, Arch. f. Hygiene, **10**.

[5] Compt. rend., **35**. and Arch. génér. de Med. (5), **2**.

into the perspiration. *Uric acid* has also been found in the perspiration in gout and *cystine* in cystinuria.

Chromhidrosis is the name given to the secretion of colored perspiration. Sometimes perspiration has been observed to be colored blue by indigo (Bizio), by pyocyanin, or by ferro-phosphate (Collmann [1]). True blood-sweat, in which blood-corpuscles exude from the opening of the glands, has also been observed.

The *exchange of gas through the skin* is of great importance for non-scaly amphibians; in mammalia, birds and human beings it is of little importance compared with the exchange of gas by the lungs. The absorption of oxygen by the skin, which was first shown by Regnault and Reiset, is small, and according to Zuelzer amounts under the most favorable circumstances to $\frac{1}{100}$ of the oxygen absorbed by the lungs. The quantity of carbon dioxide eliminated by the skin increases with the rise of temperature (Aubert, Röhrig, Fubini and Ronchi, Barratt and according to Willebrand beginning at 33°).[2] It especially increases with hyperæmia of the skin and in particular after muscular activity. It is also greater in light than in darkness. It is greater during digestion than when fasting, and greater after a vegetable than after an animal diet (Fubini and Ronchi). The quantity calculated by different investigators for the entire skin surface in twenty-four hours varies between 2.23 and 32.8 grams. According to Schierbeck and Willebrand[3] the average quantity is 7.5–9 grams, and it is ordinarily given as about 1.5 per cent of the quantity eliminated by the lungs. In a horse Zuntz, with Lehmann and Hagemann,[4] found for twenty-four hours an elimination of carbon dioxide by the skin and intestine which amounted to nearly 3 per cent of the total respiration. Less than four-fifths of this carbon dioxide came from the skin respiration. The same investigators found that the skin respiration equals 2½ per cent of the simultaneous lung respiration.

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[1] Bizio, Wien. Sitzungsber., **39**; Collmann, cited from v. Gorup-Besanez's Lehrbuch, 4. Aufl., 555.

[2] Zuelzer, Zeitschr. f. klin., Med., **53**; Aubert, Pflüger's Arch., **6**; Röhrig, Deutsch. Klin., 1872, 209; Fubini and Ronchi, Moleschott's Untersuch. z. Naturlehre, **12**; Barratt, Journ. of Physiol., **21**; Willebrand, Skand. Arch. f. Physiol., **13**.

[3] See Hoppe-Seyler, Physiol. Chem., 580; Schierbeck, Arch. f. (Anat. u.) Physiol., 1892; Willebrand, l. c.

[4] Arch. f. (Anat. u.), Physiol., 1894, and Maly's Jahresber., **24**.

CHAPTER XVII.

CHEMISTRY OF RESPIRATION.

During life a constant exchange of gases takes place between the animal body and the surrounding medium. Oxygen is inspired and carbon dioxide expired. This exchange of gases, which is called respiration, is brought about in man and vertebrates by the nutritive fluids, blood and lymph, which circulate in the body and which are in constant communication with the outer medium on one side and the tissue-elements on the other. Such an exchange of gaseous constituents may take place wherever the anatomical conditions offer no obstacle, and in man it may go on in the intestinal tract, through the skin, and in the lungs. As compared with the exchange of gas in the lungs, the exchange already mentioned, which occurs in the intestine and through the skin, is very insignificant. For this reason we will discuss in this chapter only the exchange of gas between the blood and the air of the lungs on one side and the blood and lymph and the tissues on the other. The first is often designated as external respiration, and the other, internal respiration.

I. THE GASES OF THE BLOOD.

Since the pioneer investigations of Magnus and Lothar Meyer the gases of the blood have formed the subject of repeated careful investigations by prominent experimenters, among whom must be mentioned first C. Ludwig and his pupils and E. Pflüger and his school and C. Bohr. By these investigations not only has science been enriched by a mass of facts, but also the methods themselves have been made more perfect and accurate. In regard to these methods, as also in regard to the laws of the absorption of gases by liquids, dissociation, and related questions, the reader is referred to text-books on physiology, on physics, and on gasometric analysis.

The gases occurring in blood under physiological conditions are *oxygen, carbon dioxide* and *nitrogen*, and traces of argon, and perhaps also carbon monoxide. Traces of hydrogen and marsh-gas also sometimes occur. The nitrogen is found only in very small quantities, on an average 1.2 vols. per cent. The quantity is here, as in all following experiments, calculated for 0° C. and 760 mm. pressure. The nitrogen

seems to be simply absorbed by the blood, at least in great part. It appears, like argon, to play no direct part in the processes of life, and its quantity varies but slightly in the blood of different blood-vessels.

The oxygen and carbon dioxide behave otherwise, as their quantities have significant variations, not only in the blood from different blood-vessels, but also because many factors, such as a difference in the rapidity of circulation, a different temperature, alkalinity, rest and activity cause a change. In regard to the gases they contain, the greatest difference is observable between the blood of the arteries and that of the veins.

The *quantity of oxygen* in the arterial blood of dogs is on an average 22 vols. per cent (PFLÜGER, BOHR and HENRIQUES). In human blood SETSCHENOW found about the same quantity, namely, 21.6 vols. per cent. LOEWY in another manner has determined the quantity of oxygen which the blood can take up by first shaking human venous blood with air and then calculating from this the quantity of oxygen in human arterial blood. He calculates the average amount as 18 vols. per cent. Lower figures have been found for the blood of herbivora, such as horse, sheep, rabbits and birds (hen and ducks) namely, 14–10.7 per cent (ZUNTZ and HAGEMANN, SCZELKOW, WALTER, JOLYET). Venous blood in different vascular regions has variable quantities of oxygen. By summarizing a great number of analyses by different experimenters ZUNTZ has calculated that the venous blood of the right side of the heart contains on an average 7.15 per cent less oxygen than the arterial blood.

The *quantity of carbon dioxide* in the arterial blood (of dogs) is about 40 vols. per cent (LUDWIG, SETSCHENOW, PFLÜGER, P. BERT, BOHR and HENRIQUES and others), or a little above. In herbivora and the above-mentioned birds the quantity of carbon dioxide in the arterial blood is higher than in the carnivorous dog. SETSCHENOW found 40.3 vols. per cent in human arterial blood. The quantity of carbon dioxide in venous blood varies still more (LUDWIG, PFLÜGER, and their pupils, P. BERT, MATHIEU and URBAIN, and others). According to the calculations of ZUNTZ, the venous blood of the right side of the heart contains about 8.2 per cent more carbon dioxide than the arterial. The average amount may be put down as 50 vols. per cent. HOLMGREN found in blood after asphyxiation even 69.21 vols. per cent carbon dioxide.[1]

Oxygen is absorbed only to a small extent by the plasma, whose absorbability for oxygen is 97.5 per cent of that of water, according to

[1] All the figures given above may be found in Zuntz's "Die Gase des Blutes" in Hermann's Handbuch d. Physiol., 4, Thl. 2, 33–43, which also contains detailed statements and the pertinent literature, and Bohr in Nagel's Handbuch der Physiologie des Menschen, Bd. 1, Hefte 1, 1905 and in Loewy, Handb. d. Bioch. of C. Oppenheimer, Bd. 4.

Bohr. The greater part or nearly all of the oxygen is loosely combined with the hæmoglobin. The quantity of oxygen which is contained in the blood of the dog corresponds closely to the quantity which, from the activity of the hæmoglobin, we should expect to combine with oxygen, and from the quantity of hæmoglobin contained therein. It is difficult to ascertain how far the circulating arterial blood is saturated with oxygen, as immediately after bleeding a loss of oxygen always takes place. Still it seems to be unquestionable that it is not quite completely saturated, with oxygen, in life. The laws which regulate the binding of the oxygen in the blood will be found in the discussion of the gas exchange between the blood and the air of the lungs.

The carbon dioxide of the blood occurs in part, and indeed, according to the investigations of Alex. Schmidt,[1] Zuntz,[2] and L. Fredericq,[3] to the extent of at least one-third in the blood-corpuscles, also in part, and in fact the greatest part, in the plasma or serum. Bohr[4] claims that about 30 mm. may be considered as the average pressure of the carbon dioxide in the organsim, and with such a pressure the quantity of physically dissolved CO_2 in 100 cc. of the blood amounts to 2.01 cc. As the blood with this tension takes up about 40 vols. per cent CO_2, therefore about 5 per cent of the total carbon dioxide is simply dissolved. Under the assumption that the blood corpuscles make up about $\frac{1}{3}$ of the volume of the blood, of the physically dissolved CO_2, 0.59 cc. exists with the corpuscles and 1.42 cc. with the plasma.

As the blood corpuscles in 100 cc. blood as above stated take up at the above pressure about 14 cc. CO_2, only a small part of its CO_2 is physically dissolved. The chief mass of the CO_2 is loosely combined and the constituent of these cells which unites with the CO_2 seems to be the alkali combined with phosphoric acid, oxyhæmoglobin or hæmoglobin, and globulin on one side and the hæmoglobin itself on the other. That in the red blood-corpuscles alkali phosphate occurs in such quantities that it may be of importance in the combination with carbon dioxide is not to be doubted; and it must be allowed that from the diphosphate, by a greater partial pressure of the carbon dioxide, monophosphate and alkali carbonate are formed, while by a lower partial pressure of the carbon dioxide, the mass action of the phosphoric acid again comes into play, so that, with the carbon dioxide becoming free, a reformation of alkali diphosphate takes place. It is generally admitted that the blood-coloring matters, especially the oxyhæmoglobin, which can expel carbon

[1] Ber. d. k. sächs. Gesellsch. d. Wissensch. math.-phys. Klasse, 1867.

[2] Centralbl. f. d. med. Wissensch., 1867, 529.

[3] Recherches sur la constitution du Plasma sanguin, 1878, 50, 51.

[4] In regard to the work of Bohr we will refer here and in future to Nagel's Handbuch der Physiologie des Menschen, Bd. 1.

dioxide from sodium carbonate *in vacuo*, acts like acids; and as the globulins also act similarly (see below), these bodies may also occur in the blood-corpuscles as an alkali combination. The alkali of the blood-corpuscles must, therefore, according to the law of mass action, be divided between the carbon dioxide, phosphoric acid, and the other constituents of the blood-corpuscles which possess acidic properties, and among these especially the blood pigments, because the globulin can hardly be of importance on account of its small quantity. By greater mass action or greater partial pressure of the carbon dioxide, bicarbonate must be formed at the expense of the diphosphates and the other alkali combinations, while at a diminished partial pressure of the same gas, with the escape of carbon dioxide, the alkali diphosphate and the other alkali combinations must be reformed at the cost of the bicarbonate.

Hæmoglobin must nevertheless, as the investigations of Setschenow [1] and Zuntz, and especially those of Bohr and Torup,[2] have shown, be able to hold the carbon dioxide loosely combined even in the absence of alkali. Bohr has also found that the disassociation curve of the carbon dioxide hæmoglobin corresponds essentially to the curve of the absorption of carbon dioxide, on which ground he and Torup consider the hæmoglobin itself as of importance in the binding of the carbon dioxide of the blood, and not its alkali combinations. According to Bohr the hæmoglobin takes up the two gases, oxygen and carbon dioxide, simultaneously by the oxygen uniting with the pigment nucleus and the carbon dioxide with the protein component. But as according to the researches of Zuntz [3] the combination of hæmoglobin with the alkali is first split to any great extent with a carbon dioxide tension of more than 70 mm., it must be admitted that with the ordinary CO_2 pressure in the organism, the combination of the carbon dioxide in the blood corpuscles does not essentially take place through the agency of the alkali but chiefly by means of the hæmoglobin.

The chief part of the carbon dioxide of the blood is found in the blood-plasma or the blood-serum, which follows from the fact that the serum is richer in carbon dioxide than the corresponding blood itself. By experiments with the air-pump on blood-serum it has been found that the chief part of the carbon dioxide contained in the serum is given off in a vacuum, while a smaller part can be removed only after the addition of an acid. The red blood-corpuscles also act as an acid, and therefore in blood all the carbon dioxide is expelled *in vacuo*. Hence a part of the carbon dioxide is in firm chemical combination in the serum.

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[1] Centralbl. f. d. med. Wissensch., 1877. See also Zuntz in Hermann's Handbuch, 76.

[2] Zuntz, l. c., 76; Bohr, Maly's Jahresber., 17; Torup, *ibid.*

[3] Centralbl. f. d. med. Wissensch., 1867.

Absorption experiments with blood-serum have shown us further that the carbon dioxide which can be pumped out is in great part loosely chemically combined, and from this loose combination of the carbon dioxide it necessarily follows that the serum must also contain simply absorbed carbon dioxide. For the form of binding of the carbon dioxide contained in the serum or the plasma there are the three following possibilities: 1. A part of the carbon dioxide is simply absorbed; 2. Another part is in loose chemical combination; 3. A third part is in firm chemical combination.

The quantity of physically dissolved carbon dioxide in the serum cannot be higher than about 2 vols. per cent, as the quantity of carbon dioxide in the plasma corresponding to 100 cc. of blood is given above as 1.42 cc.

The quantity of carbon dioxide in the blood-serum which is combined as a firm chemical union depends upon the quantity of simple alkali carbonate in the serum. This amount is not known, and it cannot be determined either by the alkalinity found by titration, nor can it be calculated from the excess of alkali found in the ash, because the alkali is not only combined with carbon dioxide, but also with other bodies, especially with protein. The quantity of carbon dioxide in firm chemical combination cannot be ascertained after pumping out *in vacuo* without the addition of acid, because to all appearances certain active constituents of the serum, acting like acids, expel carbon dioxide from the simple carbonate. The quantity of carbon dioxide not expelled from dog-serum by vacuum alone without the addition of acid amounts to 4.9 to 9.3 vols. per cent, according to the determinations of PFLÜGER.[1]

From the occurrence of simple alkali carbonates in the blood-serum it naturally follows that a part of the loosely combined carbon dioxide of the serum which can be pumped out must exist as bicarbonate. The occurrence of this combination in the blood-serum has also been directly shown. In experiments with the pump, as well as in absorption experiments, the serum behaves in other ways differently from a solution of bicarbonate, or carbonate of a corresponding concentration; and the action of the loosely combined carbon dioxide in the serum can be explained only by the occurrence of bicarbonate in the serum. By means of a vacuum the serum always allows much more than one-half of the carbon dioxide to be expelled, and it follows from this that in the pumping out not only may a dissociation of the bicarbonate take place, but also a conversion of the double sodium carbonate into a simple salt. As we know of no other carbon-dioxide combination, besides the bicarbonate, in the serum from which the carbon dioxide can be set free by simple

[1] E. Pflüger's Ueber die Kohlensäure des Blutes, Bonn, 1864, 11. Cited from Zuntz in Hermann's Handbuch, 65.

dissociation *in vacuo*, it must be assumed that the serum contains other weak acids, in addition to the carbon dioxide, which contend with it for the alkalies, and which expel the carbon dioxide from simple carbonates *in vacuo*. The carbon dioxide which is expelled by means of the pump, and which, without regard to the quantity merely absorbed, is generally designated as "carbon dioxide in loose chemical combination," is thus only obtained in part in dissociable loose combinations; in part it originates from the simple carbonates, from which it is expelled, *in vacuo*, by other weak acids.

These weak acids are thought to be in part phosphoric acid and in part globulins. The importance of the alkali phosphates in the carbon dioxide combination has been shown by the investigations of FERNET; but the quantity of these salts in the serum is, at least in certain kinds of blood, for example, in ox-serum, so small that it can hardly be of importance. In regard to the globulins, SETSCHENOW is of the opinion that they do not act as acids themselves, but form a combination with carbon dioxide, producing carboglobulinic acid, which unites with the alkali. According to SERTOLI,[1] whose views have found a supporter in TORUP, the globulins themselves are the acids which are combined with the alkali of the blood-serum. In both cases the globulins would form, directly or indirectly, that chief constituent of the plasma or of the blood-serum which, according to the law of mass action, contends with the carbon dioxide for the alkalies. By a greater partial pressure of the carbon dioxide the latter deprives the globulin alkali of a part of its alkali, and bicarbonate is formed; by low partial pressure carbon dioxide is set free and it is abstracted from the bicarbonate by the globulin alkali. It must also be added that the above-mentioned carboglobulinic acid can perhaps be considered as a dissociable combination of carbonic acid and protein.

The assumption that the proteins of the blood are bodies active in combining with the carbon dioxide has received some support from the investigations of SIEGFRIED[2] on the combination of carbon dioxide with amphoteric amino bodies. SIEGFRIED has found that amino-acids combine with carbon dioxide, thereby being converted into carbamino-acids (glycocoll) for example, into carbamino acetic acid, $\begin{array}{l} \qquad\qquad\quad H \\ \qquad\qquad\quad | \\ CH_2—N—COOH, \\ | \\ COOH \end{array}$ and that the carbon dioxide can be readily split off from these compounds. The peptones and serum proteins in the presence of calcium hydroxide

[1] Hoppe-Seyler, Med. chem. Untersuch., 350.

[2] Zeitschr. f. physiol. Chem., 44 and 46.

may also act in the same manner as amino-acids. Protein carbamino-acids are formed, and the possibility of such a binding of carbon dioxide must also be considered.

In the foregoing it has been assumed that the alkali is the most essential and important constituent of the blood-serum, as well as of the blood in general, in uniting with the carbon dioxide. The fact that the quantity of carbon dioxide in the blood greatly diminishes with a decrease in the quantity of alkali strengthens this assumption. Such a condition is found, for example, after poisoning with mineral acids. Thus WALTER found only 2–3 vols. per cent carbon dioxide in the blood of rabbits into whose stomachs hydrochloric acid had been introduced. In the comatose state of diabetes mellitus the alkali of the blood seems to be in great part saturated with acid combinations, β-oxybutyric acid (STADELMANN, MINKOWSKI), and MINKOWSKI[1] found only 3.3 vols. per cent carbon dioxide in the blood in diabetic coma.

GASES OF THE LYMPH AND SECRETIONS.

The gases of the lymph are the same as in the blood-serum, and the lymph stands close to the blood-serum in regard to the quantity of the various gases, as well as to the kind of carbon-dioxide combination. The investigations of DAENHARDT and HENSEN[2] on the gases of human lymph are at hand, but it still remains a question whether the lymph investigated was quite normal. The gases of normal dog-lymph were first investigated by HAMMARSTEN.[3] These gases contained traces of oxygen and consisted of 37.4–53.1 per cent CO_2 and 1.6 per cent N at 0° C. and 760 mm. Hg pressure. About one-half of the carbon dioxide was in firm chemical combination. The quantity was greater than in the serum from arterial blood, but smaller than from venous blood.

The remarkable observation of BUCHNER, that the lymph collected after asphyxiation is poorer in carbon dioxide than that of the breathing animal, is explained by ZUNTZ[4] by the formation of acid in the tissues, and especially in the lymphatic glands, immediately after death, and this acid in part decomposes the alkali carbonates of the lymph.

The secretions, with the exception of the saliva, in which PFLÜGER and KÜLZ found respectively 0.6 per cent and 1 per cent oxygen, are almost free from oxygen. The quantity of nitrogen is the same as in blood, and the chief mass of the gases consists of carbon dioxide. The quantity of this gas is chiefly dependent upon the reaction, i.e., upon the quan-

[1] Walter, Arch. f. exp. Path. u. Pharm., **7**; Stadelmann, *ibid.*, **17**; Minkowski, Mittheil a. d. med. Klinik in Königsberg, 1888.

[2] Virchow's Arch., **37**.

[3] Ber. d. k. sächs. Gesellsch. d. Wissensch., math.-phys. Klasse, **23**.

[4] Buchner, Arbeiten aus der physiol. Anstalt zu Leipzig, 1876; Zuntz, l. c., 85.

tity of alkali. This follows from the analyses of PFLÜGER. He found 19 per cent carbon dioxide removable by the air-pump and 54 per cent firmly combined carbon dioxide in a strongly alkaline bile, but, on the contrary, 6.6 per cent carbon dioxide removable by the air-pump and 0.8 per cent firmly combined carbon dioxide in a neutral bile. Alkaline saliva is also very rich in carbon dioxide. As average for two analyses made by PFLÜGER of the submaxillary saliva of a dog we have 27.5 per cent carbon dioxide removable by the air-pump and 47.4 per cent chemically combined carbon dioxide, making a total of 74.9 per cent. KÜLZ [1] found a maximum of 65.78 per cent carbon dioxide for the parotid saliva, of which 3.31 per cent was removable by the air-pump and 62.7 per cent was firmly combined. From these and other reports as to the quantity of carbon dioxide removable by the air-pump and chemically combined in the alkaline secretions it follows that bodies occur in them, although not in appreciable quantities, which are analogous to the protein bodies of the blood-serum and which act like weak acids.

The acid or at any rate non-alkaline secretions, urine and milk, contain, on the contrary, considerably less carbon dioxide, which is almost all removable by the air-pump, and a part seems to be loosely combined with the sodium phosphate. The figures found by PFLÜGER for the total quantity of carbon dioxide in milk and urine are 10 and 18.1–19.7 per cent respectively.

EWALD [2] made investigations on the quantity of gas in pathological transudates. He found only traces, or at least only very insignificant quantities of oxygen in these fluids. The quantity of nitrogen was about the same as in blood; that of carbon dioxide was greater than in the lymph (of dogs), and in certain cases even greater than in the blood after asphyxiation (dog's blood). The tension of the carbon dioxide was greater than in venous blood. In exudates the quantity of carbon dioxide, especially that firmly combined, increases with the age of the fluid, while, on the contrary, the total quantity of carbon dioxide, and especially the quantity firmly combined, decreases with the quantity of pus-corpuscles.

II. THE EXCHANGE OF GAS BETWEEN THE BLOOD, ON THE ONE HAND, AND PULMONARY AIR AND THE TISSUES, ON THE OTHER.

In the introduction (Chapter I, p. 3) it was stated that we are to-day of the opinion, derived especially from the researches of PFLÜGER and his pupils, that the oxidations of the animal body do not take place in the fluids and juices, but are connected with the form-elements and

[1] Pflüger, Pflüger's Arch., 1 and 2; Külz, Zeitschr. f. Biologie 23. It seems as if Külz's results were not calculated at 760 millimeters Hg, but rather at 1 meter.

[2] C. A. Ewald, Arch. f. (Anat. u.) Physiol., 1873 and 1876.

tissues. It is nevertheless true that oxidations take place in the blood, although only to a slight extent; but these oxidations depend, it seems, upon the form-elements of the blood, hence it does not contradict the above statement that the oxidations exclusively occur in the cells and chiefly in the tissues.

The gaseous exchange in the tissues, which has been designated internal respiration, consists chiefly in that the oxygen passes from the blood in the capillaries to the tissues, while the great bulk of the carbon dioxide of the tissues originates therein and passes into the blood of the capillaries. The exchange of gas in the lungs, which is called external respiration, consists, as is seen by a comparison of the inspired and expired air, in the blood taking oxygen from the air in the lungs and giving off carbon dioxide. This does not exclude the fact that in the lungs, as in every other tissue, an internal respiration takes place, namely, a combustion with a consumption of oxygen and formation of carbon dioxide. According to Bohr and Henriques[1] the lungs take a variable but always an important part in the total metabolism. This part, which on an average is 33 per cent, but may even rise above 60 per cent of the total metabolism, depends, these experimenters say, upon the fact that the intermediary metabolic products formed in the tissues are burnt in the lungs. It is also in part represented by secretory work of the lungs.

What kind of processes take part in this double exchange of gas? Is the gaseous exchange simply the result of an unequal tension of the blood on one side and the air in the lungs or tissues on the other? Do the gases pass from a place of higher pressure to one of a lower, according to the laws of diffusion, or are other forces and processes active?

These questions are closely related to that of the tension of the oxygen and carbon dioxide in the blood and in the air of the lungs and tissues.

Oxygen occurs in the blood in a disproportionately large part as oxyhæmoglobin, and the law of the dissociation of oxyhæmoglobin is of fundamental importance in the study of the tension of the oxygen in the blood.

Attempts have been made to prove this law by investigations on a pure solution of hæmoglobin, and Hüfner[2] has made very careful and important determinations on such solutions. Recent investigations of Bohr[3] and his pupils, as well as of Loewy and Zuntz,[4] have shown that the conditions in the blood are different from a pure hæmoglobin solution, which, in part, may be due to a change in the hæmoglobin

[1] Centralbl. f. Physiol., 6, and Maly's Jahresber., 27.

[2] Arch. f. (Anat. u.) Physiol., 1890 and 1894.

[3] See Nagel's Handbuch, and Krogh, Skand. Arch. f. Physiol., 16.

[4] Arch. f. (Anat. u.) Physiol., 1904.

brought about in its preparation. A hæmoglobin solution in which alcohol is used in preparing it, combines more firmly with oxygen than the blood, and the dissociation tension of the oxygen is greater in blood than in such a hæmoglobin solution.

The oxygen tension may be variable, as LOEWY[1] has shown, with different individuals, and it is not the same in the blood of different animals with the same oxygen pressure; for example, it is less in cat's blood than in the dog, horse and human blood. The temperature is also of great importance, as the dissociation tension increases with a rise in temperature, and with the same pressure the blood combines with less oxygen at a high temperature than at a low temperature. The influence of the concentration of the hæmoglobin manifests itself in that in dilute solutions the oxygen is more firmly combined (HÜFNER, LOEWY and ZUNTZ, BOHR) and that consequently blood made laky with water has a lower dissociation tension and a firmer binding of the oxygen than undiluted blood.

Of especial interest is the finding of BOHR, HASSELBALCH and KROGH[2] that the CO_2 present also influences the oxygen taken up, in that as the carbon dioxide tension (also within physiological limits) increases the oxygen taken up, diminishes. The laws of oxygen absorption must be determined by determinations upon blood itself, at the same time observing the temperature and the carbon dioxide tension. A series of determinations made by KROGH[3] upon horse's blood at 38° and a constant carbon dioxide tension will be given below. In calculating the results in column 4 the quantity of oxygen chemically combined at 150 mm. oxygen pressure is equal to 100.

	In 100 cc. blood		Oxygen taken up	
Oxygen Tension in mm.	Chemically combined Oxygen	Oxygen dissolved in plasma	Per cent chemically combined	Dissolved in 100 cc. plasma
10	6.0	0.020	30.0	0.030
20	12.9	0.041	64.7	0.061
30	16.3	0.061	81.6	0.091
40	18.1	0.081	90.4	0.121
50	19.1	0.101	95.4	0.152
60	19.5	0.121	97.6	0.182
70	19.8	0.141	98.8	0.212
80	19.9	0.162	99.5	0.243
90	19.95	0.182	99.8	0.273
150	20.00	0.303	100.0	0.455

From the above table we see that even with an oxygen tension which amounts to only one-half of the oxygen pressure in the air, the hæmoglobin is saturated in greatest part with oxygen. The dissociation is hence at 70-80 mm. pressure only slightly more than with a pressure of 150 mm.

[1] Arch. f. (Anat. u.) Physiol., 1904.

[2] Centralbl. f. Physiol., **17**, and Skand. Arch. f. Physiol., **16**.

[3] Skand. Arch, f. Physiol., **16**.

and indeed even with as low a pressure as 40–30 mm., still 90–80 per cent of the entire quantity of oxygen taken up chemically at 150 mm. is combined with the hæmoglobin.

From these and other observations it follows that the oxygen partial pressure may sink to one-half of that existing in the atmospheric air without markedly influencing the oxygen content of the blood. This also coincides with the experience of FRÄNKEL and GEPPERT [1] on the action of low air pressures upon the oxygen content of the blood of dogs. With an air pressure of 410 mm. Hg they found that the oxygen content of arterial blood was normal. With an air pressure of 378–365 mm. it was slightly diminished, and only on reducing the pressure to 300 mm. was a mentionable decrease observed. A. LOEWY [2] found that the lowest oxygen pressure of the alveolar air wherein the exchange of material can go on normally both qualitatively and quantitatively, is equal to 30 mm. Hg.

In regard to the above-mentioned action of low air pressure it must be remarked that the alveolar oxygen tension is regulated by changes in the respiration, so that with great diminution in the quantity of oxygen of the inspired air, the alveolar air contains the same quantity of oxygen as with a higher oxygen partial pressure of the inspired air (LOEWY). For example, LOEWY found the same quantity of oxygen, namely, 6.1 per cent, in the alveolar air with 16 and with 10.5 per cent oxygen in the inspired air, because the respiration in the latter case was 8.5 liters per minute against only 4.9 liters in the first case.

It may be concluded from the large quantity of oxygen or oxyhæmoglobin in the arterial blood that the tension of the oxygen in the arterial blood must be relatively higher. This is substantiated by the earlier observations of BERT and HÜFNER, as well as by the determinations of HERTER, FRÉDERICQ and others,[3] using aerotonometric methods, which will be mentioned below in connection with the carbon dioxide tension. HERTER found the oxygen tension in the arterial blood of dogs to be equal, on an average, to a pressure of 78.7 mm. Hg, and FRÉDERICQ, by a better method, found that it was equal to 91–99 mm. Hg.

The oxygen tension of the venous blood of dogs has been found by aerotonometric means to be equal to 20.6–27.7 mm. (STRASSBURG, FALLOISE), and by means of the lung-catheter (see below) equal to 25.5–27 mm. (WOLFBERG, NUSSBAUM). For human venous blood LOEWY and

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[1] Ueber die Wirkungen der verdünnten Luft auf den Organismus., Berlin, 1883.

[2] A. Loewy, Untersuch. über die Respiration und Zirculation, etc., Berlin, 1895; also Centralbl. f. Physiol., **13**, 449, and Arch. f. (Anat. u.) Physiol., 1900.

[3] Bert, La pression barometrique, Paris, 1878; Herter, Zeitschr. f. physiol. Chem., **3**; Hüfner, l. c.; Frédericq, Centralbl. f. Physiol., **7**, and Travaux der laborat. de l'inst. de physiol. de Liège, **5**, 1896.

v. SCHRÖTTER[1] found an average of 37.68 mm. These results do not coincide with the investigations of BOHR,[2] who in many cases obtained essentially higher figures for the oxygen tension in arterial blood.

He experimented on dogs, allowing the blood, whose coagulation had been prevented by the injection of peptone solution or infusion of the leech, to flow from one bisected carotid to the other, or from the femoral artery to the femoral vein, through an apparatus called by him an hæmataërometer. The apparatus, which is a modification of LUDWIG'S rheometer (*stromuhr*), allowed, according to BOHR, of a complete interchange between the gases of the blood circulating through the apparatus and a quantity of gas whose composition was known at the beginning of the experiment and inclosed in the apparatus. The mixture of gases was analyzed after an equalization of the gases by diffusion. In this way the tension of the oxygen and carbon dioxide in the circulating arterial blood was determined. During the experiment the composition of the inspired and expired air was also determined, the number of inspirations noted, and the extent of respiratory exchange of gas measured. To be able to make a comparison between the gas tension in the blood and in an expired air whose composition was closer to the unknown composition of the alveolar air than the ordinary expired air, the composition of the expired air at the moment it passed the bifurcation of the trachea was ascertained by special calculation. The tension of the gases in this "bifurcated air" could be compared with the tension of the gases of the blood, and in such a way that the comparison took place simultaneously. Recently KROGH[3] constructed an apparatus, called by him *microtonometer*, to be used for the same purpose.

BOHR found remarkably high results for the oxygen tension in arterial blood in this series of experiments. They varied between 101 and 144 mm. Hg pressure. In eight out of nine experiments on the breathing of atmospheric air, and in four out of five experiments on breathing air containing carbon dioxide, the oxygen tension in the arterial blood was higher than the "bifurcated air." The greatest difference, where the oxygen tension was higher in the blood than in the air of the lungs, was 38 mm. Hg.

HÜFNER and FRÉDERICQ[4] have made the objection to BOHR'S experiments and views that a perfect equilibrium had probably not been attained between the air in the apparatus and the gases of the blood. FRÉDERICQ, by new experiments, presents strong objections to the acceptance of BOHR'S findings, while on the other hand BOHR not only defends his experiments, but also finds errors in the experiments of his opponents, while HALDANE and SMITH'S[5] experiments, making use of

[1] Strassburg, Pflüger's Arch., 6; Falloise, Bull. Acad. Roy. Belg., 1902; Wolfberg, Pflüger's Arch., 4 and 6; Nussbaum, *ibid.*, 7; Loewy and v. Schrötter, cited by Loewy in Oppenheimer's Handb., 4, 76.

[2] Skand. Arch. f. Physiol., 2, and Nagel's Handbuch der Physiologie.

[3] Skand. Arch. f. Physiol., 20.

[4] Hüfner, Arch. f. (Anat. u.) Physiol. 1890; Frédericq, Centralbl. f. Physiol., 7, and Traveaux du laboratorie de l'institute de physiologie de Liège, 5, 1896.

[5] Haldane, Journ. of Physiol., 18; Haldane and Smith, *ibid.*, 20.

an entirely different principle, tend to corroborate the high results attained by BOHR.

HALDANE'S method is as follows: The individual experimented upon is allowed to inspire air containing an exactly known but small quantity of carbon monoxide (0.045–0.06 per cent), until no further absorption of carbon monoxide takes place and the percentage saturation of the hæmoglobin in the arterial blood with carbon monoxide has become constant, as shown by a special titration method. This percentage saturation is dependent upon the relation between the tension of the oxygen in the blood and the tension of the carbon monoxide, as known from the composition of the inspired air. When this last and the percentage saturation with carbon monoxide and oxygen are known the oxgyen tension in the blood can be easily calculated.

According to this method HALDANE and SMITH found still higher figures than BOHR for the oxygen tension in the blood, and they calculated the average tension of the oxygen in human arterial blood to be equal to 293 mm. Hg.

Let us now compare the figures for the oxygen tension of the arterial blood as found by various investigators with the tension of the oxygen in the air of the lungs.

Numerous investigations as to the composition of the inspired atmospheric air as well as the expired air are at hand, and it can be said that these two kinds of air at 0° C. and a pressure of 760 mm. Hg have the following average composition in volume per cent:

	Oxygen	Nitrogen (and argon)	Carbon Dioxide
Atomspheric air.	20.96	79.02	0.03
Expired air.	16.03	79.59	4.38

The partial pressure of the oxygen of the atmospheric air corresponds at a normal barometric pressure of 760 mm. to a pressure of 150 mm. Hg. The loss of oxygen which the inspired air suffers in respiration amounts to about 4.93 per cent, while the expired air contains about one hundred times as much carbon dioxide as the inspired air.

The expired air is therefore a mixture of alveolar air with the residue of inspired air remaining in the air-passages; hence in the study of the gaseous exchange in the lungs the alveolar air must first be considered. There exists no direct determination of the composition of the alveolar air in man, but only approximate calculations. From the average results found by VIERORDT in normal respiration for the carbon dioxide in the expired air, 4.63 per cent, ZUNTZ [1] has calculated the probable quantity of carbon dioxide in the alveolar air as equal to 5.44 per cent. If we start from this value, with the assumption that the quantity of nitrogen in the alveolar air does not essentially differ from the expired air, and admit that the quantity of oxygen in the alveolar

[1] See Zuntz, l. c., Hermann's Handbuch, 105 and 106.

air is 6 per cent less than the inspired air, it will be seen that the alveolar air contains 15 per cent oxygen. As the total pressure of the air of the lungs after deducting the aqueous tension of about 50 mm. can be calculated as about 710 mm. the partial pressure of the oxygen in man can be put at about 106 mm. and that of the carbon dioxide as about 45 mm.

Based upon several respiration experiments upon different persons, LOEWY has been able to calculate the composition of the alveolar air of human beings almost at the atmospheric pressure, from the composition of the expired air and the depth of inspiration and expiration, taking into consideration the air in the upper air-passages. He obtained results which varied between 101 and 105 mm. Hg for the oxygen tension and between 32–42 mm. for the carbon dioxide tension.

The alveolar oxygen tension in dogs can be calculated from the carbon dioxide content of the alveolar air and is also found to be above 100 mm. Hg.

If the oxygen partial pressure in the alveoli is put at about 105 mm. Hg, and we compare this with the highest results obtained for the oxygen tension of the arterial blood as determined by tonometric means, we find that the taking up of oxygen in the lungs can be simply explained according to physical laws as a diffusion process. The conditions are quite different if we start with the high-tension results of BOHR, 101–144 mm. Hg., or the still higher results of HALDANE and SMITH. The oxygen tension in the blood is, in many cases, according to these latter authors, always higher than the tension in the lungs, as average for various races of animals. In these cases the passage of oxygen from the lungs to the blood cannot be explained simply by a diffusion. We must therefore, with BOHR, accept a special specific activity of the lungs, and according to him a secretory activity of the lungs also exists besides diffusion.

As opinions on the taking up of oxygen are disputed so also are those on the giving up of carbon dioxide.

The tension of the carbon dioxide in the blood has been determined in different ways by PFLÜGER and his pupils, WOLFFBERG, STRASSBURG, and NUSSBAUM.[1]

According to the aerotonometric method the blood is allowed to flow directly from the artery or vein through a glass tube which contains a gas mixture of a known composition. If the tension of the carbon dioxide in the blood is greater than the gas mixture, then the blood gives up carbon dioxide, while in the reverse case it takes up carbon dioxide from the gas mixture. The analysis of the gas mixture after passing the blood through it, will also decide if the tension of the carbon dioxide in the blood is greater or less than in the gas mixture; and by a sufficiently great number of determinations, especially when the quantity of carbon dioxide of the gas mixture corresponds as closely as possible, in the beginning, to

[1] Wolffberg, Pflüger's Arch., 6; Strassburg, *ibid.*; Nussbaum, *ibid.*, 7.

the probable tension of this gas in the blood, we may learn the tension of the carbon dioxide in the blood. As above mentioned the oxygen tension can be determined by the same method.

According to this method the carbon-dioxide tension of the arterial blood is on an average 2.8 per cent of an atmosphere,[1] corresponding to a pressure of 20 mm. mercury (STRASSBURG). In the blood from the pulmonary alveoli NUSSBAUM found a carbon-dioxide tension of 3.81 per cent of an atmosphere, corresponding to a pressure of 27 mm. mercury. STRASSBURG, who experimented in non-tracheotomized dogs in which the ventilation of the lungs was less active and therefore the carbon dioxide was removed from the blood with less readiness, found in the venous blood of the heart, a carbon-dioxide tension of 5.4 per cent of an atmosphere, corresponding to a partial pressure of 38.3 mm. mercury.

Another method, which was first used by PFLÜGER and his pupils WOLFFBERG and NUSSBAUM, depends upon excluding a part of the lungs by means of the *lung catheter*.

The principle of this method is as follows: By the introduction of a catheter, of a special construction, into a branch of a bronchus the corresponding lobe of the lung may be hermetically sealed, while in the other lobes of the same lung, and in the other lung, the ventilation remains unchanged, so that no accumulation of carbon dioxide takes place in the blood. When the cutting off lasts so long that a complete equalization between the gases of the blood and the retained air of the lungs is assumed, a sample of this air of the lungs is removed by means of the catheter and analyzed.

When a complete exchange between the gases of the inclosed part of the lungs and the gases of the circulating venous blood has taken place, the tension of the gases in this part of the lungs can be considered as a measure for the gas tension in the venous blood, if we admit that the gas exchange is due only to physical forces. In their experiments WOLFFBERG and NUSSBAUM found only 3.6 per cent CO_2 in the air taken out with the catheter. NUSSBAUM also determined the carbon-dioxide tension in the blood from the right heart in a case simultaneous with the catheterization of the lungs. He found almost identical results, namely, a carbon-dioxide tension of 3.84 per cent and 3.81 per cent of an atmosphere, which also shows that complete equalization between the gases of the blood and lungs in the inclosed parts of the lungs had taken place. The method of catheterizing the lungs is, as shown by LOEWY and v. SCHRÖTTER,[2] also applicable to man, and they found that the carbon dioxide tension of human venous blood was equal to 6 per cent

[1] Here and in the following discussion we mean by atmospheric pressure the pressure in the lungs after subtracting the aqueous vapor tension (about 50 mm.), namely, 760 − 50 = 710 mm. mercury pressure.

[2] l. c., footnote 1, page 812.

of the atmospheric pressure in the lungs=42.6 mm.Hg, while according to LOEWY'S calculations the carbon dioxide tension in the respired lung alveoli varied between 31.8 and 41.8 mm. Hg with an average of 37.3 mm. Hg for eleven cases.

According to these investigations the giving up of carbon dioxide may also be explained by physical laws; but BOHR, in his experiments above mentioned (page 812), has arrived at other results in regard to the carbon-dioxide tension. In eleven experiments with inhalation of atmospheric air the carbon-dioxide tension in the arterial blood varied from 0 to 38 mm. Hg, and in five experiments with inhalation of air containing carbon dioxide, form 0.9 to 57.8 mm. Hg. A comparison of the carbon-dioxide tension in the blood with the bifurcated air gave in several cases a greater carbon-dioxide pressure in the air of the lungs than in the blood, and as maximum this difference amounted to 17.2 mm. in favor of the air of the lungs in the experiments with inhalation of atmospheric air. As the alveolar air is richer in carbon dioxide than the bifurcated air this experiment unquestionably proves, according to BOHR, that the carbon dioxide has migrated against the high pressure.

In opposition to these investigations, FRÉDERICQ,[1] in his above-mentioned experiments, obtained the same figures for the carbon-dioxide tension in arterial peptone blood as PFLÜGER and his pupils found for normal blood. WEISGERBER,[2] in FRÉDERICQ'S laboratory has made experiments with animals which respired air rich in carbon dioxide, and these experiments confirm PFLÜGER'S theory of respiration. Recently FALLOISE has made determinations of the carbon-dioxide tension of venous blood by means of FRÉDERICQ'S aërotonometer. The carbon-dioxide tension was found to equal 6 per cent of an atmosphere, hence somewhat higher than the results found by PFLÜGER'S pupils. In opposition to these investigations BOHR has presented strong objections; he has demonstrated the principles for the construction of the tonometer, and claims that the earlier experiments with the tonometer are not conclusive, as a complete equilibrium of the gas tension was not attained.

A certain importance has been ascribed to oxygen in regard to the elimination of carbon dioxide in the lungs, in that it has an expelling action on the carbon dioxide from its combinations in the blood. This theory, first advanced by HOLMGREN, has recently found an advocate in WERIGO. Still ZUNTZ has presented weighty objections to WERIGO'S experiments, and BOHR[3] has later also shown that we have no positive basis for the above assumption.

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[1] See footnote 3, page 811.

[2] Centralbl. f. Physiol. **10**, 482; Falloise, see Maly's Jahresber, **32**.

[3] Holmgren. Wien. Sitzungsber., 48 Werigo, Pflüger's Arch., **51** and **52**; Zuntz, *ibid.*, **52**; Bohr, see Nagel's Handbuch der Physiologie.

The conditions as to the elimination of carbon dioxide in the lungs are not quite clear, and from the above we see that in regard to the gas exchange in the lungs we have two opposing theories. According to the older view suggested by the PFLÜGER school the exchange of gas follows simple physical laws and is on the whole a diffusion process. According to BOHR'S opinion diffusion does take place, but the lung is a gland which has the power of secreting gases, and the gas exchange in the lungs is essentially a secretory process.

The theory that a secretion of carbon dioxide takes place in the lungs has been further supported by recent investigations by BOHR.[1]

One group of experiments includes those carried out, essentially, according to an earlier principle, but using the microtonometer of KROGH. The other group includes those experiments where each lung breathed by itself, the one atmospheric air and the other a gas mixture containing a considerable quantity of carbon dioxide, namely, about 8 per cent. The first group of experiments gave new support for the secretion theory, but the second group were of still greater interest. In these experiments—in which the alveolar air of both lungs had a different quantity of carbon dioxide, for example in one about 3 per cent CO_2 and the other about 9 per cent, while the circulating blood was the same in the two separated respiring lungs (right heart blood)—the carbon dioxide tension in the venous blood was always lower than in the air of the CO_2 respiring lung, and nevertheless CO_2 was eliminated in this lung. This cannot be explained by the diffusion theory; and in the opinion of HAMMARSTEN it cannot be disputed that the investigations thus far reported seem to support BOHR'S theory strongly, and this is also corroborated by the secretion of gases detected in certain animals.

That a true secretion of gases occurs in animals follows from the composition and behavior of the gases in the swimming-bladder of fishes. These gases consist of oxygen and nitrogen with only small quantities of carbon dioxide. In fishes which do not live at any great depth the quantity of oxygen is ordinarily as high as in the atmosphere, while fishes which live at great depths may, according to BIOT and others, contain considerably more oxygen and even above 80 per cent. MOREAU has also found that after emptying the swimming-bladder by means of a trocar, new air collected after a time, and this air was richer in oxygen than the atmospheric air, and contained even 85 per cent oxygen. BOHR, who has proven and confirmed these statements, also found that this accumulation is under the influence of the nervous system, because on the section of certain branches of the pneumogastric nerve it is discontinued. It is beyond dispute that there is here a secretion and not a diffusion of oxygen. Recently JAEGER [2] has given a further explanation as to the secretory activity of the swimming-bladder.

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[1] Centralbl. f. Physiol., **21**, 367.

[2] Biot, see Hermann's Handbuch d. Physiol., **4**, Thl. 2, 151; Moreau, Compt. rend., **57**; Bohr, Journ. of Physiol., **15**. See also Hüfner, Arch. f. (Anat. u.) Physiol., 1892; Jaeger, Pflüger's Arch., **94**.

From what has been said above (page 809) in regard to the internal respiration, one can conclude that it consists chiefly that in the capillaries the oxygen passes from the blood into the tissues, while the carbon dioxide passes from the tissues into the blood.

The assertion of Estor and Saint Pierre that the quantity of oxygen in the blood of the arteries decreases with the remoteness from the heart has been shown to be incorrect by Pflüger,[1] and the oxygen tension in blood on entering the capillaries must be higher. The oxygen tension of the plasma is of importance in the giving up of oxygen to the tissues, as the blood corpuscles contain a supply of oxygen only sufficient to replace that removed from the plasma by the tissue. This quantity of oxygen, which is dissolved in the plasma and at the disposal of the tissues, is dependent upon the oxygen tension in the blood and only indirectly dependent upon the total quantity of oxygen in the blood. As this tissue is almost or entirely free from oxygen, a considerable difference in regard to the oxygen pressure must exist between the blood and the tissues. The possibility that this difference in pressure is sufficient to supply the tissues with the necessary quantity of oxygen is hardly to be doubted.

The animal body, it seems, also has the command over means of regulating and varying the oxygen tension, and such a means is the carbon dioxide produced in the tissue which, according to Bohr, Hasselbach, and Krogh,[2] raises the oxygen tension. This is of special importance when the tension of the oxygen in the blood of the capillaries is very low; then the ability of the carbon dioxide to raise the dissociation tension of the oxyhæmoglobin comes into play, especially with low oxygen tension. Another regulating moment is, Bohr claims, the *specific oxygen capacity* of the blood, which means the relation of the maximum oxygen combination to the quantity of iron of the blood or the hæmoglobin solution.

As the hæmoglobin prepared from different blood portions does not, according to Bohr, always take up the same quantity of oxygen for each gram, so the hæmoglobin within the blood-corpuscle may show a similar behavior. He calls the quantity of oxygen (measured at 0° C. and 760 mm. Hg) which is taken up by 1 gram of hæmoglobin of the blood at 15° C. and an oxygen pressure of 150 mm. the *specific oxygen capacity*.[3] This quantity, he claims, may vary not only in different individuals, but also in the different vascular systems of the same animal, and it may also be changed experimentally by bleeding, breathing air deficient in oxygen, or poisoning. It is now evident that one and the same quantity of oxygen in the blood, other things being equal, must have a different tension according as the specific oxygen capacity is greater or smaller. The tension of the oxygen, Bohr says, may be changed without changing the quantity of oxygen, and the animal body must have means of varying the tension of the oxygen

[1] Estor and Saint Pierre with Pflüger in Pflüger's Arch., **1**.

[2] Centralbl. f. Physiol., **17**, and Skand. Arch. f. Physiol., **16**.

[3] Centralbl. f. Physiol., **4**, and Nagel's Handb. d. Physiol.

in the tissues in a short time without changing the quantity of oxygen contained in the blood. The importance for respiration of such a property of the tissues is evident; but it is perhaps too early to give a positive opinion on BOHR's statements and experiments.

In regard to the carbon-dioxide tension in the tissue it must be assumed *à priori* that it is higher than in the blood. This is found to be true. STRASSBURG [1] found in the urine of dogs and in the bile a carbon-dioxide tension of 9 per cent and 7 per cent of an atmosphere, respectively. The same experimenter has, further, injected atmospheric air into a ligatured portion of the intestine of a living dog and analyzed the air taken out after some time. He found a carbon-dioxide tension of 7.7 per cent of an atmosphere. The carbon-dioxide tension in the tissues is considerably greater than in the venous blood, and there is no opposition to the view that the carbon dioxide simply diffuses from the tissues into the blood according to the law of diffusion.

Several methods have been suggested for the study of the quantitative relation of the respiratory exchange of gas. The reader must be referred to other text-books for details as to these methods, and we will here mention only the chief features of the most important methods. It must also be remarked, in regard to these methods, that those of REGNAULT and REISET and of PETTENKOFER, determine the total gas exchange, and indeed for a long time, while the three other methods determine the respiratory gas exchange alone, and this only for a short time.

REGNAULT and REISET'S *Method.* According to this method the animal or person experimented upon is allowed to respire in an inclosed space. The carbon dioxide is removed from the air, as it forms, by strong caustic alkali, from which the quantity may be determined, while the oxygen is replaced continually by exactly measured quantities. This method, which also makes possible a direct determination of the oxgyen used as well as the carbon dioxide produced, has since been modified by other investigators, such as PFLÜGER and his pupils, SEEGEN and NOWAK, and HOPPE-SEYLER, ROSENTHAL and OPPENHEIMER and especially by ATWATER and BENEDICT.[2]

PETTENKOFER'S *Method.* According to this method the individual to be experimented upon breathes in a room through which a current of atmospheric air is passed. The quantity of air passed through is carefully measured. As it is impossible to analyze all the air made to pass through the chamber, a small fraction of this air is diverted into a branch line during the entire experiment, carefully measured, and the quantity of carbon dioxide and water determined. From the composition of this air the quantity of water and carbon dioxide contained in the large quantity of air made to pass through the chamber can be calculated. The consumption of oxygen cannot be directly determined in this method, but may be calculated indirectly by difference, which is a defect in this

[1] Pflüger's Arch., **6**.

[2] See Zuntz in Hermann's Handbuch, **4**, Thl. 2, and Hoppe-Seyler, Zeitschr. f. physiol. Chem., **19**; Rosenthal, Arch. f. (Anat. u.) Physiol., 1902; Zuntz and Oppenheimer, Arch. f. (Anat. u.) Physiol., 1905, and Bioch. Zeitschr., **14**; Atwater and Benedict, Bull, Dept. of Agriculture, Washington, 69, 109, and 136. See also Krogh, Wien. Sitz. Ber., **115**, III., and Skand. Arch. f. Physiol., **18**.

method. The large respiration apparatus of SONDÉN and TIGERSTEDT as well as of ATWATER and ROSA [1] are based upon this principle.

SPECK'S *Method.*[2] For briefer experiments on man SPECK used the following: He breathes through a mouthpiece with two valves, closing the nose with a clamp, into two spirometer-receivers, where the gas-volume can be read off very accurately. The air from one of the spirometers is inhaled through one valve and the expired air passes through the other into the other spirometer. By means of a rubber tube connected with the expiration-tube an accurately measured part of the expired air may be passed into an absorption-tube and analyzed.

ZUNTZ and GEPPERT S *Method.*[3] This method, which has been improved by ZUNTZ and his pupils from time to time, consists in the following: The individual being experimented upon inspires pure atmospheric air through a very wide feed-pipe leading from the open air, the inspired and the expired air being separated by two valves (human subjects breathe with closed nose by means of a soft-rubber mouthpiece, animals through an air-tight tracheal canula). The volume of the expired air is measured by a gas-meter and an aliquot part of this air collected and the quantity of carbon dioxide and oxygen determined. As the composition of the atmospheric air can be considered as constant within a certain limit, the production of carbon dioxide as well as the consumption of oxygen may be readily calculated (see the works of ZUNTZ and his pupils).

HANRIOT and RICHET'S *Method* [4] is characterized by its simplicity. These investigators allow the total air to pass through three gasometers, one after the other. The first measures the inspired air, whose composition is known. The second gasometer measures the expired air, and the third the quantity of the expired air after the carbon dioxide has been removed by a suitable apparatus. The quantity of carbon dioxide produced and the oxygen consumed can be readily calculated from these data.

APPENDIX

THE LUNGS AND THEIR EXPECTORATIONS.

Besides *proteins* and the *albuminoids* of the connective-substance group, *lecithin*, *taurine* (especially in ox-lungs), *uric acid*, and *inosite* have been found in the lungs. POULET [5] claims to have found a special acid in the lung-tissue, which he has called *pulmotartaric acid.* Glycogen occurs abundantly in the embryonic lung, but is absent in the adult organ. The proteolytic enzymes also belong to the physiological constituents of the lungs. They are active in the autolysis of the lungs (JACOBY) as well as in the solution of pneumonic infiltrations (FR. MÜLLER[6]).

The lungs have a strong reducing property, which BOHR explains by the extensive oxidation processes in the lungs. According to N. SIEBER

[1] Pettenkofer's method; see Zuntz, l. c.; Sondén and Tigerstedt, Skand. Arch. f. Physiol., **6**; Atwater and Rosa, Bull. of Dept. of Agriculture, **63**. Washington.

[2] Speck, Physiologie des menschlichen Atmens. Leipzig, 1892.

[3] Pflüger's Arch., **42**. See also Magnus-Levy in Pflüger's Arch., **55**, 10, in which the work of Zuntz and his pupils is cited.

[4] Compt. rend., **104**.

[5] Cited from Maly's Jahresber., **18**, 248.

[6] Jacoby, Zeitschr. f. physiol. Chem., **33**; Müller, Verhandl. d. Kongress. f. inn. Medizin, 1902.

they also have the ability to decompose neutral fats, while, RIEHL [1] says, they do not have the ability to invert milk sugar.

The black or dark-brown pigment in the lungs of human beings and domestic animals consists chiefly of carbon, which originates from the soot in the air. The pigment may in part also consist of melanin. Besides carbon, other bodies, such as iron oxide, silicic acid, and clay, may be deposited in the lungs, being inhaled as dust.

Among the bodies found in the lungs under pathological conditions must be specially mentioned, proteoses (and peptones?) in pneumonia and suppuration, glycogen, a slightly dextrorotatory carbohydrate differing from glycogen, found by POUCHET in consumptives, and finally also cellulose, which, according to FREUND,[2] occurs in the lungs, blood, and pus of persons with tuberculosis.

C. W. SCHMIDT found in 1000 grams of mineral bodies from the normal human lung the following: NaCl 130, K_2O 13, Na_2O 195, CaO 19, MgO 19, Fe_2O_3 32, P_2O_5 485, SO_3 8, and sand 134 grams. According to OIDTMANN,[3] the lungs of a 14-day old child contained 796.05 p. m. water, 198.19 p. m. organic bodies, and 5.76 p. m. inorganic bodies.

The sputum is a mixture of the mucous secretion of the respiratory passages, of saliva and buccal mucus. Because of this its composition is variable, especially under pathological conditions when various products mix with it. The chemical constituents are, besides the mineral substances, chiefly mucin with a little proteid and nuclein substance. Under pathological conditions proteoses and peptones (?), which are probably produced by bacterial action or by autolysis (WANNER, SIMON [4]), volatile fatty acids, glycogen, CHARCOT'S crystals, and also crystals of cholesterin, hæmatoidin, tyrosine, fat and fatty acids, triple phosphates, etc., have been found.

The form constituents are, under physiological circumstances, epithelium-cells of various kinds, leucocytes, sometimes also red blood-corpuscles and various kinds of fungi. In pathological conditions elastic fibers, spiral formations consisting of a mucin-like substance, fibrin coagulum, pus, pathogenic microbes of various kinds and the above-mentioned crystals occur.

The lung concretions contain chiefly calcium and phosphoric acid as inorganic constituents. Silicic acid is, in ZICKGRAF'S opinion, an essential and constant constituent, but according to GERHARTZ and STRIGEL [5] is not always constant.

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[1] N. Sieber, Zeitschr. f. physiol. Chem., **55**; Riehl, Zeitschr. f. Biol., **48**.

[2] Pouchet, Compt. rend., **96**; Freund, cited from Maly's Jahresber, **16**, 471.

[3] Schmidt, cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 727; Oidtmann, *ibid.*, 732.

[4] Wanner, Deutsch. Arch. f. klin. Med., **75**; Simon, Arch. f. exp. Path. u. Pharm., **49**.

[5] Gerhartz and Strigel, Beitr. z. klin. d. Tuberkulose, **10**, which also cites Zickgraf.

CHAPTER XVIII.

METABOLISM WITH VARIOUS FOODS, AND THEIR NECESSITY TO MAN.

I. EXCHANGE OF MATTER AND FORCE IN GENERAL WITH METHODS OF STUDY.

The conversion of chemical energy into heat and mechanical work which characterizes animal life, leads, as previously stated in Chapter I, to the formation of relatively simple compounds—carbon dioxide, urea, etc.—which leave the organism, and which, moreover, being very poor in energy, are for this reason of little or no value to the body. It is therefore absolutely necessary for the continuance of life and the normal course of the functions of the body that the organism and its different tissues should be supplied with new material to replace that which has been exhausted. This is accomplished by means of food. Those bodies are designated as *food* which have no injurious action upon the organism and which serve as a source of energy and can replace those constituents of the body that have been consumed in metabolism or that can prevent or diminish the consumption of such constituents.

Among the numerous dissimilar substances which man and animals take with the food all cannot be equally necessary or have the same value. Some perhaps are unnecessary, while others may be indispensable. We have learned by direct observation and a wide experience that besides the oxygen, which is necessary for oxidation, the essential foods for animals in general, and for man especially, are *water, mineral bodies, proteins, carbohydrates,* and *fats.*

It is also apparent that the various groups of foodstuffs necessary for the tissues and organs must be of varying importance; thus, for instance, water and the mineral bodies have another value than the organic foods, and these again must differ in importance among themselves. The knowledge of the action of various nutritive bodies on the exchange of material from a qualitative as well as a quantitative point of view must be of fundamental importance in determining the value of different nutritive substances relative to the demands of the body for food under various conditions, and also in deciding many other questions—for instance, the proper nutrition for an individual in health and in disease.

Such knowledge can be attained only by a series of systematic and thorough observations, in which the quantity of nutritive material, rela-

tive to the weight of the body, taken and absorbed in a given time is compared with the quantity of final metabolic products which leave the organism at the same time. Researches of this kind have been made by investigators, but above all should be mentioned those made by BISCHOFF and VOIT, by PETTENKOFER and VOIT, and by VOIT and his pupils, by RUBNER, ZUNTZ and by ATWATER.

It is absolutely necessary in researches on the exchange of material to be able to collect, analyze, and quantitatively estimate the excreta of the organism, so that they may be compared with the quantity and composition of the nutritive bodies ingested. In the first place, one must know what the habitual excreta of the body are and in what way these bodies leave the organism. One must also have trustworthy methods for their quantitative estimation.

The organism may, under physiological conditions, be exposed to accidental or periodic losses of valuable material—such losses as occur only in certain individuals, or in the same individual only at a certain period; for instance, the secretion of milk, the production of eggs, the ejection of semen or menstrual blood. It is therefore apparent that these losses can be the subject of investigation and estimation only in special cases.

The regular and constant excreta of the organism are of the very greatest importance in the study of metabolism. To these belong, in the first place, the true final metabolic products—*carbon dioxide, urea* (uric acid, hippuric acid, creatinine, and other urinary constituents), and a part of the *water*. The remainder of the water, the mineral bodies, and those secretions or tissue constituents—*mucus, digestive fluids, sebum, perspiration,* and *epidermal formations*—which are either poured into the intestinal tract, or secreted from the surface of the body, or broken off and thereby lost to the body, also belong to the constant excreta.

The remains of food, sometimes indigestible, sometimes digestible but not acted upon, which are contained in the feces, and which vary considerably in quantity and composition with the nature of the food, also belong to the excreta of the organism. Even though these remains, which are never absorbed and therefore are never constituents of the animal fluids or tissues, cannot be considered as excreta of the body in a strict sense, still their quantitative estimation is absolutely necessary in certain experiments on the exchange of material.

The determination of the constant loss is in some cases accompanied with the greatest difficulties. The loss from the detached epidermis, from the secretion of the sebaceous glands, etc., cannot be determined with exactness without difficulty, and therefore—as they do not occasion any appreciable loss because of their small quantity—they need not be considered in quantitative experiments on metabolism. This also applies to the constituents of the mucus, bile, pancreatic and intestinal juices, etc., occurring in the contents of the intestine, and which, leaving the body with the feces, cannot be separated from the other contents of the intestine and therefore cannot be quantitatively determined separately. The uncertainty which, because of the intimated difficulties, attaches itself to the results of the experiment, is very small as compared to the variation which is caused by different individualities, different modes of living, different foods, etc. Only approximate values can therefore be given for the constant excreta of the human body.

The following figures represent the quantity of excreta for twenty-four hours from a grown man, weighing 60–70 kilos, on a mixed diet. The numbers are compiled from the results of different investigators:

	Grams.
Water	2500–3500
Salts (with the urine)	20–30
Carbon dioxide	750–900
Urea	20–40
Other nitrogenous urinary constituents	2–5
Solids in the excrement	20–50

These total excreta are approximately divided among the various excretions in the following way; but still it must not be forgotten that this division may vary to a great extent under different external circumstances: By *respiration* about 32 per cent, by the *evaporation from the skin* 17 per cent, with the *urine* 46–47 per cent, and with the *excrement* 5–9 per cent. The elimination by the skin and lungs, which is sometimes differentiated by the name "*perspiratio insensiblis*" from the visible elimination by the kidneys and intestine, is on an average about 50 per cent of the total elimination. This proportion, quoted only relatively, is subject to considerable variation, because of the great difference in the loss of water through the skin and kidneys under varying circumstances.

The nitrogenous constituents of the excretions consist chiefly of urea, or uric acid in certain animals, and the other nitrogenous urinary constituents. A disproportionately large part of the nitrogen leaves the body with the urine, and, as the nitrogenous constituents of this excretion are final products of the metabolism of proteins in the organism, the quantity of proteins catabolized in the body may be easily calculated by multiplying the quantity of nitrogen in the urine by the coefficient 6.25 ($\frac{100}{16} = 6.25$), if it is admitted that the proteins contain in round numbers 16 per cent of nitrogen.

Still another question is whether the nitrogen leaves the body only with the urine or by other channels. The latter is habitually the case. The discharges from the intestine always contain some nitrogen, which as stated in Chapter IX consists in part of non-absorbed remnants of the food, but in chief part and sometimes entirely of constituents of the epithelium and the secretions. Under these circumstances it is apparent that one cannot give any exact figures which are valid for all cases for that part of the nitrogen of the excrement which originates in the digestive tract and in the digestive fluids. It may not only vary in different individuals, but also in the same individual after more or less active secretion and absorption. In the attempts made to determine this part of the nitrogen of the excrement it has been found that in man, on non-nitrogenous or nearly nitrogen-free food, it amounts in round numbers

to somewhat less than 1 gram per twenty-four hours (RIEDER, RUBNER). Even with such food the absolute quantity of nitrogen eliminated by the feces increases with the quantity of food because of the accelerated digestion (TSUBOI [1]), and is greater than in starvation. MÜLLER [2] found in his observations on the faster CETTI that only 0.2 gram nitrogen was derived from the intestinal canal.

The quantity of nitrogen which leaves the body under normal circumstances by means of the hair and nails, with the scaling off of the skin, and with the perspiration cannot be accurately determined. It is nevertheless so small that it may be ignored. Only in profuse sweating need the elimination by this channel be taken into consideration.

The view was formerly held that in man and carnivora an elimination of gaseous nitrogen took place through the skin and lungs, and because of this, on comparing the nitrogen of the food with that of the urine and feces, a *nitrogen deficit* occurred in the visible elimination.

This question has been the subject of much discussion and of numerous investigations, the most recent by KROGH and OPPENHEIMER.[3] These researches have shown that the above assumption is unfounded, and moreover several authorities, especially PETTENKOFER and VOIT, and GRÜBER,[4] have shown by experiments on man and animals that with the proper quantity and quality of food the body can be brought into *nitrogenous equilibrium*, in which the quantity of nitrogen voided with the urine and feces is equal or nearly equal to the quantity contained in the food. Undoubtedly we must admit, with VOIT, that a deficit of nitrogen does not exist, or it is so insignificant that in experiments upon metabolism it need not be considered. Ordinarily, in investigations on the catabolism of proteins in the body, it is only necessary to consider the nitrogen of the urine and feces, but it must be remarked that the nitrogen of the urine is a measure of the extent of the catabolism of the proteins in the body, while the nitrogen of the feces (after deducting about 1 gram on a mixed diet) is a measure of the non-absorbed part of the nitrogen of the food. The nitrogen of the food, as well as of the excreta, is generally determined by KJELDAHL'S method.

In the oxidation of the proteins in the organism their sulphur is oxidized into sulphuric acid, and on this depends the fact that the elimination of sulphuric acid by the urine, which in man is but to a small extent derived

[1] Rieder, Zeitschr. f. Biologie, **20**; Rubner, *ibid.*, **15**; Tsuboi, *ibid.*, **35**.

[3] Berlin. klin. Wochenschr., 1887.

[2] See Regnault and Reiset, Annal. d. chem. et phys. (3), **26**, and Annal. d. Chem. u. Pharm., **73**; Seegen and Nowak, Wien. Sitzungsber., **71**, and Pflüger's Arch., **25**; Pettenkofer and Voit, Zeitschr. f. Biologie, **16**; Leo, Pflüger's Arch., **26**; Krogh, Skand. Arch. f. Physiol., **18**, and Wien. Sitz. Ber., **115**, III: Oppenheimer, Bioch. Zeitschr., **4**.

[4] Pettenkofer and Voit, in Hermann's Handbuch, **6**, Thl. 1; Grüber, Zeitschr. f. Biologie, **16** and **19**.

from the sulphates of the food, makes nearly equal variations with the elimination of nitrogen by the urine. If the amount of nitrogen and sulphur in the proteins is considered as 16 per cent and 1 per cent respectively, then the proportion between the nitrogen of the proteins and the sulphuric acid, H_2SO_4, produced by their combustion is in the ratio 5.2:1, or about the same as in the urine (see page 726). The determination of the quantity of sulphuric acid eliminated in the urine gives us an important means of controlling the extent of the transformation of proteins, and such a control is especially important in cases in which it is expected to study the action of certain nitrogenous non-albuminous bodies on the metabolism of proteins. A determination of the nitrogen alone is not sufficient in such cases. A perfectly positive measure of the protein catabolism cannot be made from the sulphuric acid of the urine, as the various protein substances have a rather variable sulphur content, and on the other hand also a variable quantity of the sulphur in the urine exists as so-called neutral sulphur.

In metabolism experiments the total sulphur of the urine as well as the feces must be determined, and it may also be of importance to determine the relation between the sulphuric acid-sulphur and the neutral sulphur of the urine. The sulphur of the catabolized proteins is more quickly eliminated, according to v. Wendt, Hämäläinen and Helme [1] than the nitrogen, and this behavior of sulphur gives a more positive picture of the temporal catabolism of protein than the nitrogen. This is of importance, as the elimination of the nitrogen corresponding to a certain amount of protein requires several days for completion. Falta has also observed that the chief amount of nitrogen in man on taking different proteins is secreted with varying rapidity, and the same is true, according to Hämäläinen and Helme, for the elimination of sulphur, as in their experiments the sulphur elimination from white of egg required about six days and from casein only two days. These conditions must be considered in metabolism experiments.

Besides lecithins and other phosphatides the body takes with its food pseudonucleins as well as true nucleins, and these are absorbed more or less completely from the intestinal tract and then assimilated (Gumlich, Sandmeyer, Marcuse, Röhmann, and Steinitz, Loewi,[2] and others).

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[1] Wendt, Skand. Arch. f. Physiol., **17**; Hämäläinen and Helme, *ibid.*, **19**; Falta, Deutsch. Arch. f. klin. Med., **86**.

[2] In regard to the investigations on the metabolism of phosphorus and the methods used therein, see Steinitz, Pflüger's Arch., **72**; Zadik, *ibid.*, **77**; Leipziger, *ibid.*, **78**; Oertel, Zeitschr., f. physiol. Chem., **26**; Mandel and Oertel, Bull. Med. Sciences, N. Y. Univ., **1**, and Ehrlich, Inaug.-Diss., Breslau, 1900; Loewi, Arch, f. exp. Path. u. Pharm., **45**; Slowtzoff, Hofmeister; Beiträge 8. On the absorption of casein, see Poda, Prausnitz, Micko, and P. Müller, Zeitschr. f. Biologie, **39**. The literature on the phosphorus

On the other hand, the phosphorized protein substances, lecithins and phosphatides, are also decomposed within the body, and their phosphorus is chiefly eliminated as phosphoric acid and also in part as organic phosphorus (see page 718). For these reasons the phosphorus is of great importance in certain investigations on metabolism.

If it is found, on comparing the nitrogen of the food with that of the urine and feces, that there is an excess of the first, this means that the body has increased its stock of nitrogenous substances—proteins. If, on the contrary, the urine and feces contain more nitrogen than the food taken at the same time, this denotes that the body is giving up part of its nitrogen—that is, part of its own proteins has been decomposed.

We can, from the quantity of nitrogen, as above stated, calculate the corresponding quantity of proteins by multiplying by 6.25.[1] Usually, according to Voit's proposition, the nitrogen of the urine is not calculated as decomposed proteins, but as decomposed muscle-substance or flesh. Lean meat contains on an average about 3.4 per cent nitrogen; hence each gram of nitrogen of the urine corresponds in round numbers to about 30 grams of flesh. The assumption that lean meat contains 3.4 per cent nitrogen is arbitrary, and the relation of N : C in the proteins of dried meat, which is of great importance in certain experiments on metabolism, is given differently by various experimenters, namely, 1 : 3.22–1 : 3.68. Argutinsky found in beef, after complete removal of fat and subtraction of glycogen, that the relation was 1 : 3.24 (see Chapter XI).

The carbon leaves the body chiefly as carbon dioxide, which is eliminated by the lungs and skin. The remainder of the carbon is excreted in the urine and feces in the form of organic compounds, in which the quantity of carbon can be determined by elementary analysis. It was formerly considered sufficient to calculate the quantity of carbon in the urine from the quantity of nitrogen according to the relation N : C = 1 : 0.67. This does not seem to be trustworthy, as this relation varies and depends, according to Tangl and Pflüger, Langstein, and Steinitz,[2] upon the kind of food. Tangl has shown that the richer the food is in carbohydrates the more carbon and hence the more heat of combustion per gram of nitrogen does the urine contain. He found the following for 1 gram of nitrogen in the urine: With diet rich in fat 0.747 gram C and 9.22 calories; for carbohydrate-rich diet he found 0.936 gram C and 11.67 calories.

The extent of the gas exchange can be determined by any of the methods given on pages 819, 820. By multiplying the quantity of carbon dioxide found by 0.273 one obtains the quantity of carbon eliminated as CO_2.

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metabolism can be found in Albu and Neuberg, Physiol. u. Pathol. des Mineralstoffwechsels, Berlin, 1906.

[1] In calculating the protein catabolism from the nitrogen of the urine it must not be forgotten that the food often contains nitrogenous extractives whose nitrogen cannot be calculated as protein and for which a special correction must be made, if necessary.

[2] Tangl, Arch. f. (Anat. u.) Physiol., 1899, Supplbd.; Pflüger in Pflüger's Arch., **79**; Langstein and Steinitz, Centralbl. f. Physiol., **19**.

If the total quantity of carbon eliminated in various ways is compared with the carbon contained in the food some idea can be obtained as to the transformation of the carbon compounds. If the quantity of carbon in the food is greater than in the excreta, then the excess is deposited; while if the reverse be the case it shows a corresponding loss of body substance.

The nature of the substances here deposited or lost, whether they consist of proteins, fats, or carbodydrates, is learned from the total quantity of the nitrogen of the excretions. The corresponding quantity of proteins may be calculated from the quantity of nitrogen, and, as the average quantity of carbon in the proteins is known, the quantity of carbon which corresponds to the decomposed proteins may be easily ascertained. If the quantity of carbon thus found is smaller than the quantity of the total carbon in the excreta, it is then obvious that some other nitrogen-free substance has been consumed besides the proteins. If the quantity of carbon in the proteins is considered in round numbers as 52.5 per cent, then the relation between carbon (52.5) and nitrogen (16) is 3.28, or in round numbers 3.3:1. If the total quantity of nitrogen eliminated is multiplied by 3.3, the excess of carbon in the eliminations over the product found represents the carbon of the decomposed non-nitrogenous compounds. For instance, in the case of a person experimented upon, 10 grams of nitrogen and 200 grams of carbon were eliminated in the course of twenty-four hours; then these 62.5 grams of protein correspond to 33 grams of carbon, and the difference, $200-(3.3\times10)=167$, represents the quantity of carbon in the decomposed non-nitrogenous compounds. If we start from the simplest case, starvation, where the body lives at the expense of its own substance, then, since the quantity of carbohydrates as compared with the fats of the body is extremely small, in such cases in order to avoid mistakes the assumption must be made that the person experimented upon has used only fat and proteins. As animal fat contains on an average 76.5 per cent carbon, the quantity of transformed fat may be calculated by multiplying the carbon by $\frac{100}{76.5}=1.3$. In the case of the above example, the person experimented upon would have used 62.5 grams of proteins and $167\times1.3=217$ grams of fat of his own body in the course of the twenty-four hours.

Starting from the nitrogen balance, it can be calculated in the same way whether an excess of carbon in the food as compared with the quantity of carbon in the excreta is retained by the body as proteins or fat or as both. On the other hand, with an excess of carbon in the excreta one can determine how much of the loss of the substance of the body is due to a consumption of the proteins on the one side and of non-nitrogenous bodies on the other side. How to especially calculate the part taken by the fats and carbohydrate will be shown in connection with the calculation of the energy metabolism.

The quantity of water and mineral bodies voided with the urine and feces can easily be determined. The quantity of water eliminated by the skin and lungs may be directly estimated by means of the large respiration apparatus.

The organic constituents of the body as well as the foodstuffs introduced, represent a sum of chemical energy which the body can use for force. The exchange of material is also an exchange of force, and the first stands in such close relation to the second that the study of one cannot be separated from the other. The energy theory of metabolism has

exercised an extraordinarily fruitful influence upon the entire study of metabolism and nutrition, and this is due in great measure to the work of RUBNER.

This energy of the various foods may be represented by the amount of heat which is set free in their combustion. This quantity of heat is expressed as calories, and a small calorie is the quantity of heat necessary to warm 1 gram of water from 0° to 1° C. A large calorie is the quantity of heat necessary to warm 1 kilo of water 1° C. Here and in the following pages large calories are to be understood. There are numerous investigations by different experimenters, such as FRANKLAND, DANILEWSKI, RUBNER, BERTHELOT, STOHMANN, BENEDICT and OSBORNE, and others, on the calorific value of different foodstuffs. The following results, which represent the calorific value of a few nutritive bodies on complete combustion outside of the body to the highest oxidation products, are taken from STOHMANN'S [1] work.

	Calories.
Casein	5.86
Ovalbumin	5.74
Conglutin	5.48
Protein (average)	5.71
Animal tissue-fat	9.50
Butter-fat	9.23
Cane-sugar	3.96
Milk-sugar	3.95
Dextrose	3.74
Starch	4.19

Fats and carbohydrates are completely burnt in the body, and one can therefore consider their combustion equivalent as a measure of the living force developed by them within the organism. We generally designate 9.3 and 4.1 calories for each gram of substance as the average for the physiological calorific value of fats and carbohydrates respectively.

The proteins act differently from the fats and carbohydrates. They are only incompletely burnt, and they yield certain decomposition products, which, leaving the body with the excreta, still represent a certain quantity of energy which is lost to the body. The heat of combustion of the proteins is smaller within the organism than outside of it, and they must therefore be specially determined. For this purpose RUBNER [2] fed a dog on washed meat, and he subtracted from the heat of combustion of the food the heat of combustion of the urine and feces, which corresponded to the food taken plus the quantity of heat necessary for the swelling up of the proteins and the solution of the urea. RUBNER has also tried to determine the heat of combustion of the proteins (muscle-

[1] See Rubner, Zeitschr. f. Biologie, **21**, which also cites the works of Frankland and Danilewski; see also Berthelot, Compt. rend., **102**, **104**, and **110**; Stohmann, Zeitschr. f. Biologie, **31**; Benedict and Osborne (vegetable proteins), Journ. of biol. Chem., **3**.

[2] Zeitschr. f. Biologie, **21**.

proteins) decomposed in the body of rabbits in starvation. According to these investigations, the physiological heat of combustion in calories for each gram of substance is as follows:

1 gram. of the dry substance.	Calories.
Protein from meat	4.4
Muscle	4.0
Protein in starvation	3.8
Fat (average for various fats)	9.3
Carbohydrates (calculated average)	4.1

The physiological combustion value of the various foods belonging to the same group is not quite the same. It is, for instance, 3.97 calories for a vegetable protein, conglutin, and 4.42 calories for an animal protein body, syntonin. According to RUBNER the normal heat value per 1 gram of animal protein may be considered as 4.23 calories, and of vegetable protein as 3.96 calories. When a person on a mixed diet takes about 60 per cent of the proteins from animal foods and about 40 per cent from vegetable foods, the value of 1 gram of the protein of the food is equivalent to about 4.1 calories. The physiological value of each of the three chief groups of organic foods, by their decomposition in the body, is in round numbers as follows:

	Calories.
1 gram protein	4.1
1 gram fat	9.3
1 gram carbohydrate	4.1
1 gram alcohol	7.1

These figures are generally used in the calculation of the energy content of various foodstuffs and diets.

The extent of gas exchange and the so-called respiratory quotient is, besides the extent of nitrogen elimination, of the greatest importance in the calculation of the extent of energy metabolism and the division of the energy between the protein, fat and carbohydrate.

On comparing the inspired and the expired air we learn, on measuring them when dry and at the same temperature and pressure, that the volume of the expired air is less than that of the inspired air. This depends upon the fact that not all of the oxygen appears again in the expired air as carbon dioxide, because it is not only used in the oxidation of carbon, but also in part in the formation of water, sulphuric acid, and other bodies. The volume of expired carbon dioxide is regularly less than the volume of the inspired oxygen, and the relation $\frac{CO_2}{O}$, which is called the *respiratory quotient,* is generally less than 1.

The magnitude of the respiratory quotient is dependent upon the kind of substances destroyed in the body. In the combustion of pure carbon one volume of oxygen yields one volume of carbon dioxide, and the quotient is therefore equal to 1. The same is true in the burning of

carbohydrates, and in the exclusive decomposition of carbohydrates in the animal body the respiratory quotient must be approximately 1. In the exclusive metabolism of proteins it is close to 0.80, and with the decomposition of fat it is 0.7. In starvation, as the animal draws on its own flesh and fat, the respiratory quotient must be a close approach to the latter figure. The respiratory quotient, which is calculated with exclusive combustion of carbohydrate, fat and protein, as respectively, 1, 0.707 and 0.809 and with alcohol is 0.667, also gives important information as to the quality of material decomposed in the body, especially with the supposition that the carbon dioxide elimination is not influenced by some special condition such as a change in the respiratory mechanism. Another supposition is that no incomplete oxidation step in combustion is eliminated.

Knowledge as to the extent of oxygen consumption is of special importance in the calculation of the energy metabolism from the extent of gas exchange, and one can under some circumstances approximately calculate the energy exchange from the calorific value of the oxygen alone—with regard to the respiratory quotient (ZUNTZ and co-workers). The calorific value of oxygen must be different for each of the three mentioned foodstuffs, as they require different quantities of oxygen for their combustion. For fat and carbohydrate this calorific value can be readily calculated, as bodies are completely burnt into carbon dioxide and water. One gram of starch uses 828.8 cc. oxygen in its combustion, and produces 828.8 cc. carbon dioxide, and 4183 calories of heat are developed. For one liter (=1.43 gram) oxygen, 5047 calories are produced, therefore for every liter (=1.966 gram) carbon dioxide formed, the same number, 5047 calories, are produced. In an analogous manner the average calorific value of fat for 1 liter of oxygen, 4686 calories, and for 1 liter carbon dioxide, 6629 calories, can be calculated.

With proteins, because of the unequal composition of the different proteins, the results are uncertain and variable, and the calculation is much more complicated. As example we will give the following calculation of ZUNTZ [1] for the fat-free dry substance of meat.

This substance consisted in 100 parts

	52.38g.C.;	7.27g.H.;	22.68g.O.;	16.65g.N.;	1.02g.S.
Of which were found in the urine	9.406	2.663	14.099	16.28	1.02
Of which were found in the feces	1.471	0.212	0.889	0.37	
Retained	41.50;	4.40;	7.69;	0.0;	0.0.

From this residue, with the taking up of 96.63 liters of oxygen, besides 39.6 grams water, 77.39 liters carbon dioxide were formed and the respiratory quotient is therefore 0.801. Now 100 grams of such dry meat substance on complete

[1] Zuntz, Loewy, Müller and Caspari, Höhenklima und Bergwanderungen, Berlin, 1006, pages 102, 103

combustion yields 563.09 calories, and if we subtract the calorific value of the corresponding urine (=113.70 calories) and feces (=17.76 calories), the sum, 131.46 calories, then 431.63 calories were set free in the body. For every gram of nitrogen eliminated in the urine (16.28 gram.) there is produced $\frac{431.63}{16.28}=26.51$ calories; the corresponding quantity of oxygen is $\frac{96.63}{16.28}=5.91$ liter O and the corresponding quanity of CO_2 produced is $\frac{77.39}{16.28}=4.75$ liters CO_2. The calorific value for 1 liter of oxygen consumed is therefore $\frac{26.51}{5.91}=4.485$ calories, and for 1 liter of carbon dioxide produced $\frac{26.51}{4.75}=5.579$ calories.

For milk protein ZUNTZ has calculated for 1 gram urea nitrogen 5.8 liters oxygen, 4.6 liters carbon dioxide and 27 calories. The calorific value can be calculated from this for 1 liter O=4.66 and for 1 liter CO_2=5.87 calories. If we take the average of these calculations we obtain a calorific value for the combustion of protein, which for 1 liter of oxygen amounts to 4.573 calories and for the carbon dioxide 5.725 calories.

According to these calculations the combustion of proteins in the animal body has a calorific value for 1 liter of oxygen of, in round numbers, 4.57 calories, and for 1 liter CO_2, 5.73 calories. We therefore have the following calorific values for the three foodstuffs:

	Per 1 liter Oxygen.	Relative value.	Per 1 liter Carbon dioxide.	Relative value.
Protein	4.57	100	5.73	113.4
Fat	4.69	102.6	6.63	131.3
Carbohydrate	5.05	110.5	5.05	100.0

The figures for the oxygen vary less than those for the carbon dioxide, and this is a reason why the oxygen values are better suited than the CO_2 values for calculating the energy production from the extent of gas exchange. Other investigators have obtained results which correspond more or less with the above values for the heat value of oxygen, and E. VOIT and KUMMACHER,[1] who have made calculations in another way, have obtained still smaller differences for the relative oxygen value.

From what was said above we can calculate the extent of protein metabolism, the corresponding development of energy and the corresponding absorption of oxygen and carbon dioxide formation from the quantity of nitrogen in the urine. If we subtract the oxygen and carbon dioxide values from the total, directly determined gas exchange, the result represents the fats and carbohydrates used. According to ZUNTZ from this residue we can calculate the heat value of the oxygen used as well as the division of the decomposition of the fat and carbohydrate by considering the respiratory quotient. For this purpose ZUNTZ and SCHUMBURG have

[1] Voit, Zeitschr. f. Biol., 44; Kummacher, *ibid.*

constructed a table, an abstract of which we give below, taken from the work of MAGNUS-LEVY.[1]

R. Q.	Calories value per 1 liter 0.	Division in per cent. Carbohydrate.	Fat.
1.000	5.047	100	0
0.950	4.986	83	17
0.900	4.924	66	34
0.850	4.863	49	51
0.800	4.801	32	68
0.750	4.740	15	85
0.707	4.686	0	100

As the calorific oxygen values in the combustion of protein, fat and carbohydrate show no great differences among themselves, in those cases where, as in starvation, the part taken by the proteins in the total metabolism is relatively small, one can calculate the total energy exchange, without any striking error, from the respiratory quotient and the oxygen used. This is especially important in experiments of short duration where the protein metabolism cannot be directly determined, but is calculated from the nitrogen elimination occurring during a longer time. The method of ZUNTZ and GEPPERT, mentioned on page 820, has shown itself especially useful in the study of the material and force exchange in these experiments of short duration, while the respiration apparatus constructed on PETTENKOFER'S or REGNAULT-REISET principle are only useful in experiments over a longer period.

KAUFMANN [2] incloses the individual to be experimented upon in a capacious sheet-iron room, which serves both as a respiration-chamber and a calorimeter, and which permits the estimation of the nitrogen of the urine and the carbon dioxide expired, as well as the inspired oxygen and the quantity of heat produced. If we start from the theoretically calculated formulæ for the various possible transformations of the proteins, fats, and carbohydrates in the body, it is clear that other values must be obtained for the heat, carbon dioxide, oxygen, and nitrogen of the urine, when one, for example, admits of a complete combustion of proteins to urea, carbon dioxide, and water, or of a partial splitting off of fat. Another relation between heat, carbon dioxide, and oxygen is also to be expected when the fat is completely burnt or when it is decomposed into sugar, carbon dioxide, and water. In this way, by a comparison of the values found in special cases with the figures calculated for the various transformations, KAUFMANN attempts to explain the various decomposition processes in the body under different nutritive conditions.

As will be shown, the fats and carbohydrates may decrease the metabolism of proteins in the body, while, on the other hand, the quantity of proteins in the body or in the food acts on the metabolism of fat in the body. In physiological combustion the various foods may replace one another to a certain extent, and it is therefore important to know the ratio of replacement. The investigations made by RUBNER have taught

[1] A. Magnus-Levy in v. Noorden's Handb. d. Pathol. des Stoffwechsels, Bd. 1. (1906).
[2] Arch. d. Physiologie (5), 8.

that this, if it relates to the force and heat production in the animal body, is a proportion that corresponds with the figures of the heat value of the same. This is apparent from the following table. In this is found the weight of the various foods equal to 100 grams of fat, a part determined from experiments on animals and a part calculated from figures of the heat values:

	100 grams fat are equal to or isodynamic with		
	From Experiments on Animals.	From the Heat Value.	Difference, per cent.
Syntonin	225	213	+5.6
Muscle-flesh (dried)	243	235	+4.3
Starch	232	229	+1.3
Cane-sugar	234	235	-0
Dextrose	256	255	−0

From the given *isodynamic value* of the various foods it follows that these substances replace one another in the body almost in exact ratio to the energy contained in them. Thus in round numbers 227 grams of protein and carbohydrate are equal to or isodynamic with 100 grams of fat in regard to source of energy, because each yields 930 calories on combustion in the body.

By means of recent very important calorimetric investigations, RUBNER[1] has shown that the heat produced in an animal in several series of experiments extending over forty-five days corresponded to within 0.47 per cent of the physiological heat of combustion calculated from the decomposed body and foods. ATWATER and his collaborators[2] have made some very thorough investigations on this subject on men. In their experiments they made use of a large respiration calorimeter, which not only exactly determined the excreta, but also made a calorimetric determination of the heat given out by the person experimented upon, i.e., the work performed. From the results of these experiments they found an almost absolutely complete agreement between the calories found directly and those calculated.

This isodynamic law is of fundamental value in the study of metabolism and nutrition. The quantity of energy in the transformed foods or the constituents of the body may be used as a measure for the total consumption of energy, and the knowledge of the quantity of energy in the foods must also be the basis for the calculation of dietaries for human beings under various conditions.

The heat value of a foodstuff can be directly determined in a calorimeter, but may also be calculated from its composition. If one subtracts from the gross heat value of the food obtained in one way or another,

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[1] Zeitschr. f. Biologie, **30**.

[2] Bull. of Dept. of Agric., Washington, **44**, **63**, **69**, and **109** and Ergebnisse des Physiologie, **3**.

the combustion heat of the feces and urine with the same diet, there is obtained the net calorific value of the diet. This value, calculated in percentage of the total energy content of the food, is called the *physiological availability* by RUBNER.[1] In order to elucidate this we will give a few of RUBNER's values. The loss in calories, as well as the physiological availability, is calculated in percentages of the total energy content of the food.

Food.	Loss in per cent. In Urine.	Loss in per cent. In the Feces.	Total loss in per cent.	Availability in per cent.
Cow's milk	5.13	5.07	10.20	89.8
Mixed diet (rich in fat)	3.87	5.73	9.60	90.4
Mixed diet (poor in fat)	4.70	6.00	10.70	89.3
Potatoes	2.00	5.60	7.60	92.4
Graham bread	2.40	15.50	17.90	82.1
Rye bread	2.20	24.30	26.50	73.5
Meat	16.30	6.90	23.20	76.8

In order to simplify the calculation of the energy exchange there exists, besides the above-mentioned standard figures for the physiological calorific value of the organic foodstuffs, also for the carbon of the carbon dioxide, and for the oxygen other standard factors. Thus for 1 gram of meat (dry substance) free from fat and extractives we have the calculated value of 5.44–5.77 calories. KÖHLER [2] found 5.678 calories for 1 gram of ash and fat-free dried-meat substance of the ox and 5.599 calories for horse meat. According to FRENTZEL and SCHREUER [3] 45.4 calories is calculated for 1 gram of nitrogen in fat and ash-free dried-meat feces (dog), while 6.97 to 7.45 calories is calculated for 1 gram of nitrogen in meat-urine. The calorific urine quotient $\frac{\text{calories}}{\text{N}}$ seems still, as above given, not to be constant for human beings, but is dependent upon the variety of food.

Instead of the direct determination the heat of combustion can also be determined from the elementary composition acording to the following principle as suggested by E. VOIT.[4] If we designate the heat of combustion for 1 gram of the substance by calories and the quantity of oxygen necessary for the complete combustion of 1 gram of the substance (=oxygen capacity of the substance) by O, then $\frac{\text{calories}}{\text{O}} = K$, which is the combustion value for 1 gram of oxygen. The oxygen capacity can be calculated from the elementary composition, and when the value of K is known, the combustion heat of a chemical compound or a known mixture can be readily determined. The value K is almost constant for substances of the same groups; but also different groups show among themselves only slight deviation for this value. VOIT obtained the following values for a few of the foodstuffs:

	K (in kg. Calories.)	O Capacity.
Plant protein	3.298	1.740
Animal protein	3.273	1.741
Fat	3.271	2.863
Carbohydrate	3.525	1.156

These methods of calculation are, according to VOIT and KRUMMACHER, admissible for practical purposes.

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[1] Zeitschr. f. Biologie, **42**.

[2] Zeitschr. f. physiol. Chem., **31**.

[3] The works of Frentzel and Schreuer may be found in Arch. f. (Anat. u.) Physiol., 1901, 1902, and 1903.

[4] Zeitschr. f. Biologie, **44**. See also Krummacher, *ibid.*

II. METALBOLISM IN STARVATION AND WITH INSUFFICIENT NUTRITION.

In starvation the decomposition in the body continues uninterruptedly, though with decreased intensity; but, as it takes place at the expense of the substance of the body, it can continue for a limited time only. When an animal has lost a certain fraction of the mass of the body, death is the result. This fraction varies with the condition of the body at the beginning of the starvation period. Fat animals succumb when the weight of the body has sunk to one-half of the original weight. Otherwise, according to CHOSSAT,[1] animals die as a rule when the weight of the body has sunk to two-fifths of the original weight. The period when death occurs from starvation not only varies with the varied nutritive condition at the beginning of starvation, but also with the more or less active exchange of material. This is more active in small and young animals than in large and older ones, but different classes of animals show an unequal activity. Children succumb in starvation in 3–5 days after having lost one-fourth of their body mass. Grown persons may, as observed upon SUCCI,[2] and other professional fasters, starve for twenty days or more without lasting injury; and there are reports of cases of starvation extending over a period of even more than forty to fifty days. Dogs can live without food from four to eight weeks, birds five to twenty days, snakes and frogs more than half a year or a whole year.

In starvation the *weight of the body* decreases. The loss of weight is greatest in the first few days, and then decreases rather uniformly. In small animals the absolute loss of weight per day is naturally less than in larger animals. The relative loss of weight—that is, the loss of weight of the unit of the weight of the body, namely, 1 kilo—is, on the contrary, greater in small animals than in larger ones. The reason for this is that the smaller animals have a greater surface of body in proportion to their mass than larger animals, and the greater loss of heat caused thereby must be replaced by a more active consumption of material.

It follows from the decrease in the weight of the body that the absolute extent of metabolism must diminish in starvation. If, on the contrary, the extent of the metabolism is referred to the unit of the weight of the body, namely, 1 kilo, it appears that this quantity remains almost unchanged during starvation. The investigations of ZUNTZ, LEHMANN, and others[3] on the professional faster CETTI showed on the third and sixth days of starvation an average consumption of 4.65 cc. oxygen per kilo in one minute, and on the ninth to eleventh day an average of 4.73 cc. The calories, as a measure of the metabolism, fell on the first to

[1] Cited from Voit in Hermann's Handbuch, 6, Thl. 1, 100.

[2] See Luciani, Das Hungern. Hamburg u. Leipzig, 1890.

[3] Berlin. klin. Wochenschr., 1887.

fifth day of starvation from 1850 to 1600 calories, or from 32.4 to 30 per kilo, and it remained nearly unchanged, if referred to the unit of body weight.[1] In man the average daily energy consumption in starvation amounts to about 30–32 calories per kilo.

The *extent of the metabolism of proteins*, or the elimination of nitrogen by the urine, which is a measure of the same, diminishes as the weight of the body diminishes. This decrease is not regular or the same during the entire period of starvation, and the extent depends, as the experiments made upon carnivora have shown, upon several circumstances. During the first few days of starvation the excretion of nitrogen is greatest, and the richer the body is in protein, due to the food previously taken, the greater is the protein catabolism or the nitrogen elimination, according to VOIT. The nitrogen elimination diminishes the more rapidly—that is, the curve of the decrease is more sudden—the richer in proteins the food was which was taken before starvation. This condition is apparent from the following table of data of three different starvation experiments made by VOIT[2] on the same dog. This dog received 2500 grams of meat daily before the first series of experiments, 1500 grams of meat daily before the second series, and a mixed diet relatively poor in nitrogen before the third series.

Day of Starvation.	Grams of Urea Eliminated in Twenty-four Hours.		
	Ser. I.	Ser. II.	Ser. III.
First	60.1	26.5	13.8
Second	24.9	18.6	11.5
Third	19.1	15.7	10.2
Fourth	17.3	14.9	12.2
Fifth	12.3	14.8	12.1
Sixth	13.3	12.8	12.6
Seventh	12.5	12.9	11.3
Eighth	10.1	12.1	10.7

In man and also in animals sometimes a rise in the nitrogen excretion is observed about the second or third starvation day, which is then followed by a regular diminution. This rise is explained by PRAUSNITZ, TIGERSTEDT, LANDERGRÉN,[3] as follows: At the commencement of starvation the protein metabolism is reduced by the glycogen still present in the body. After the consumption of the glycogen, which takes place in great part during the first days of starvation, the destruction of proteins increases as the glycogen action decreases, and then decreases again when the body has become poorer in available proteins.

Other conditions, such as varying quantities of fat in the body, have an influence on the rapidity with which the nitrogen is eliminated during

[1] See also Tigerstedt and collaborators in Skand. Arch. f. Physiol., 7.

[2] See Hermann's Handbuch. 6, Thl. 1, 89.

[3] Prausnitz, Zeitschr. f. Biologie, 29; Tigerstedt and collaborators, l. c.; Landergren, Undersökningar öfver menniskans ägghviteomsättning, Inaug.-Diss. Stockholm, 1902.

the first days of starvation. After the first few days of starvation the elimination of nitrogen is more uniform. It may diminish gradually and regularly until the death of the animals or there may be a rise in the last days, a so-called premortal increase. Whether the one or the other occurs depends upon the relation between the protein and fat content of the body.

Like the destruction of proteins during starvation, the *decomposition of fat* proceeds uninterruptedly, and the greatest part of the calories needed during starvation are supplied by the fats. According to RUBNER and VOIT the protein catabolism varies only slightly in starving animals at rest and at an average temperature, and forms a constant portion of the total exchange of energy; of the total calories in dogs 10–16 per cent comes from the protein decomposition and 84–90 per cent from the fats. This is at least true for starving animals which had a sufficiently great original fat content. If on account of starvation the animal has become relatively poorer in fat and the fat content of the body has fallen below a certain limit, then in order to supply the calories necessary a larger quantity of protein is destroyed and the premortal increase now occurs (E. VOIT). The reason for this premortal rise in protein catabolism is still not completely understood (SCHULZ and collaborators [1]).

Since the fat has a diminishing influence on the destruction of proteins corresponding to what was said above, the elimination of nitrogen in starvation is less in fat than in lean individuals. For instance, only 9 grams of urea were voided in twenty-four hours during the later stages of starvation by a well-nourished and fat person suffering from disease of the brain, while I. MUNK found that 20–29 grams urea were voided daily by CETTI,[2] who had been poorly nourished.

The investigations on the *exchange of gas* in starvation have shown, as previously mentioned, that its absolute extent is diminished, but that when the consumption of oxygen and elimination of carbon dioxide are calculated on the unit weight of the body, 1 kilo, this quantity quickly sinks to a minimum and then remains unchanged, or, on the continuation of the starvation, may actually rise. It is a well-known fact that the body temperature of starving animals remains almost constant, without showing any appreciable decrease, during the greater part of the starvation period. The temperature of the animal first sinks a few days before death, which occurs at about 33–30° C.

From what has been said about the respiratory quotient it follows that in starvation it is about the same as with fat and meat exclusively as food, i.e., approximately 0.7. This is often the case, but it may occa-

[1] Zeitschr. f. Biologie, **41**, 167 and 502. See also Kaufmann, *ibid.*, and N. Schulz, *ibid.*, and Pflüger's Arch., **76**, with Mangold, Stübel and Hempel, *ibid.*, **114**.

[2] Berl. klin. Wochenschr., 1887.

sionally be lower, 0.65–0.50, as observed in the cases of CETTI and SUCCI. This can be explained by an elimination of acetone bodies by the urine; a part can be accounted for perhaps by a formation and deposition of glycogen from protein.

Water passes uninterruptedly from the body in starvation even when none is taken. If the quantity of water in the tissues rich in proteins is considered as 70–80 per cent, and the quantity of proteins in them 20 per cent, then for each gram of protein destroyed about 4 grams of water are set free. This liberation of water from the tissues is generally sufficient to supply the loss of water, and starvation is ordinarily not accompanied with thirst. Starving animals, as a rule, do not partake of water.

The loss of water calculated on the percentage of the total organism must naturally be essentially dependent upon the previous amount of fatty tissue in the body. If we bear these conditions in mind, then, it seems, according to BÖHTLINGK,[1] that, from experiments upon white mice, the animal body is poorer in water during inanition. The body loses more water than is set free by the destruction of the tissues.

The *mineral substances* leave the body uninterruptedly in starvation until death, and the influence of the destruction of tissues is plainly perceptible by their elimination. Because of the destruction of tissues rich in potassium the proportion between potassium and sodium in the urine changes in starvation, so that, contrary to the normal conditions, the potassium is eliminated in proportionately greater quantities.

Contrary to the above BÖHTLINGK with starving white mice, and KATSUYAMA [2] with starving rabbits found a greater excretion of sodium than potassium.

MUNK observed, in CETTI's case, an increase in the elimination of phosphoric acid in relation to the *N*-elimination, which indicates an increased decomposition of bone-substance, and this explanation is supported by the fact that a simultaneous increase in the elimination of lime and magnesia occurs. Recently WELLMANN[3] showed that in rabbits, the increase in the elimination of phosphorus, calcium and magnesium in starvation corresponds to the loss in the bones of these constituents.

The question as to the participation of the different organs in the loss of weight of the body during starvation is of special interest. In elucidation of this point we give the following results of CHOSSAT's experiments on pigeons, and those of VOIT [4] on a male cat. The results are percentages of weight lost from the original weight of the organ.

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[1] Arch. des sciences biol. de St. Pétersbourg, **5**.

[2] Böhtlingk, l. c.; Katsuyama, Zeitschr. f. physiol. Chem., **26**.

[3] Munk, Berl. klin. Wochenschr., 1887; Wellmann, Pflüger's Arch., **121**.

[4] Cited from Voit in Hermann's Handbuch, **6**, Part 1, 96 and 97.

	Pigeon (Chossat).	Male Cat (Voit).
Adipose tissue	93 per cent.	97 per cent.
Spleen	71 "	67 "
Pancreas	64 "	17 "
Liver	52 "	54 "
Heart	45 "	3 "
Intestine	42 "	18 "
Muscles	42 "	31 "
Testicles	— "	40 "
Skin	33 "	21 "
Kidneys	32 "	26 "
Lungs	22 "	18 "
Bones	17 "	14 "
Nervous system	2 "	3 "

The total quantity of blood, as well as the quantity of solids contained therein, decreases, as Panum and others [1] have shown, in the same proportion as the weight of the body. Concerning the loss of water by different organs authorities disagree, Lukjanow [2] claiming that the various organs differ from each other in this respect.

The above-tabulated results cannot serve as a measure of the metabolism in the various organs during starvation. For instance, the nervous system shows only a small loss of weight as compared with the other organs, but from this it must not be concluded that the exchange of material in this system of organs is least active. The conditions may be quite different; for one organ may derive its nutriment during starvation from some other organ and exist at its expense. A positive conclusion cannot be drawn in regard to the activity of the metabolism in an organ from the loss of weight of that organ in starvation. Death by starvation is not the result of the death of all the organs of the body, but it depends more likely upon the disturbance in the nutrition of a few less vitally important organs (E. Voit [3]).

In calculating or determining the loss of weight of the organs in starvation the original fat content of the organs must be considered. With the consideration of the fat content of the organs, determined or estimated in a special way before the starvation period and at the end, E. Voit [4] found the following loss of weight in the supposed fat-free organs in starvation, namely, muscles 41 per cent, viscera 42 per cent, skin 28 per cent, and skeleton 5 per cent.

The quantity of urine nitrogen sinks in starvation corresponding to the protein catabolism, but to a varying degree in different individuals. The lowest values observed thus far in man was 2.82 grams per diem as found by E. and O. Freund on the twenty-first day in the faster Succi. Calculated on 1 kilogram of body weight the urine nitrogen, as is to be

[1] Panum, Virchow's Arch., **29**; London, Arch. d. scienc. biol. de St. Pétersbourg, **4**.
[2] Zeitschr. f. physiol. Chem., **13**.
[3] Zeitschr. f. Biologie, **41**.
[4] *Ibid.*, **46**.

expected, shows striking differences in different persons; in CETTI and SUCCI it was 0.150–0.200 gram on the fifth to tenth day of starvation. The division of the nitrogen in the urine in starvation is unlike that in the normal condition. The relative amount of urea diminishes, as shown by E. and O. FREUND, BRUGSCH and CATHCART,[1] so that instead of being about 85 per cent of the total nitrogen under normal conditions it can sink to 54 per cent (BRUGSCH). At the same time because of the abundant formation of acetone bodies (starvation acidosis) the quantity of ammonia increases considerably (BRUGSCH, CATHCART). A relative increase in the neutral sulphur of the urine also takes place (BENEDICT, CATHCART[2]).

One must differentiate between the real starvation metabolism and the metabolism in the inanition condition, the *basal requirement* (MAGNUS-LEVY) or the *maintenance value* (LOEWY[3]). With this we understand the metabolism in uniform, medium temperature, with absolute bodily rest and inactivity of the intestinal canal. As a measure of this we determine the gas exchange in a person lying down with as perfect complete muscular rest as possible, or sleeping in the early morning and at least twelve hours after a light meal not rich in carbohydrates. This basal requirement is the measure of the energy necessary for the performance of all the functions necessary to maintain life during rest; and all work above this minimum activity is called *productive increase* by MAGNUS-LEVY. The basal requirement is almost constant for the same individual and serves as the starting point in the study of the action of different influences such as work, food, diseased conditions, etc., upon metabolism. The extent of this basal requirement, as determined by the gas exchange according to the ZUNTZ-GEPPERT method,[4] and by JOHANSSON[5] and collaborators amounts in men of 60–70 kilos body weight to about 220–250 cc. oxygen and 160–200 cc. carbon dioxide per minute, which equals 20–24 grams carbon dioxide per hour. JOHANSSON found in forced complete muscular rest 20.7 gram CO_2 per hour and 24.8 gram CO_2 in the ordinary resting. According to MAGNUS-LEVY the total daily metabolism can be calculated for the basal requirement as 1625 calories, or including the rise due to the partaking of food as 1800 calories.

The food may be quantitatively insufficient, and the final result of

[1] E. and O. Freund, Wien. klin. Rundschau, 1901, Nos. 5 and 6; Brugsch, Zeitschr. f. exp. Path. u. Therap., **1** and **3**; Cathcart, Bioch. Zeitschr., **6**.

[2] Zeitschr. f. klin. Med., **36**; Cathcart, l. c.

[3] Magnus-Levy in v. Noorden's Handbuch, and Loewy in Oppenheimer's Handbuch d. Biochemie, Bd. 4.

[4] The literature can be found in the works of Magnus-Levy and Loewy.

[5] Skand. Arch. f. Physiol., **7**, **8**, **21**, and Nord. Med. Arch. Festband, 1897; see also Magnus-Levy.

this is absolute inanition. The food may also be qualitatively insufficient or, as we say, inadequate. This occurs when any of the necessary nutritive bodies are absent in the food, while the others occur in sufficient or perhaps even in excessive amounts.

Lack of water in the food. The quantity of water in the organism is greatest during fœtal life and then decreases with increasing age. Naturally, the quantity differs essentially in different organs. The enamel, with only 2 p. m. water, is the tissue poorest in water while the teeth contain about 100 p. m. and the fatty tissue 60–120 p. m. water. The bones, with 140–440 p. m., and the cartilage with 540–740 p. m. are somewhat richer in water, while the muscles, blood and glands, with 750 to more than 800 p. m., are still richer. The quantity of water is even greater in the animal fluids (see preceding chapter), and the adult body contains in all about 630 p. m. water.[1] It follows from what has been given in Chapter I in regard to the great importance of water for living processes, that, if the loss of water is not replaced by fresh supply, the organism must succumb sooner or later. Death occurs indeed sooner from lack of water than from complete inanition (Landauer, Nothwang).

If water is withdrawn for a certain time, as Landauer and especially Straub have shown, it has an accelerating influence upon the decomposition of protein. This increased destruction has, according to Landauer, the purpose of replacing a part of the water removed, by the production of water by means of the increased metabolism. The deprivation of water for a short time may, according to Spiegler,[2] especially in man, cause a diminution in the protein metabolism by means of a reduced protein absorption.

Lack of Mineral Substances in the Food. In the previous chapters attention has repeatedly been called to the importance of the mineral bodies and also to the occurrence of certain mineral substances in certain amounts in the various organs. The mineral content of the tissues and fluids is not very great as a rule. With the exception of the skeleton, which contains as average about 220 p. m. mineral bodies (Volkmann[3]), the animal fluids or tissues are poor in inorganic constituents, and the quantity of these, amounts, as a rule, only to about 10 p. m. Of the total quantity of mineral substances in the organism, the greatest part occurs in the skeleton, 830 p. m., and the next greatest in the muscles, about 100 p. m. (Volkmann).

The mineral bodies seem to be partly dissolved in the fluids and partly combined with organic substances, but nothing definite can be given as

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[1] See Voit, in Hermann's Handbuch, 6, part 1, 345.

[2] Landauer, Maly's Jahresber., 24; Nothwang, Arch. f. Hyg., 1892; Straub, Zeitschr. f. Biol., 37 and 38; Spiegler, *ibid.*, 41.

[3] See Hermann's Handbuch., 6, pt. 1. 353.

to the kind of combination, or whether they occur in stoichiometric proportions, or whether they are simply adsorption combinations. In accordance with this the organism persistently retains, with food poor in salts, a part of the mineral substances, also such as are soluble, as the chlorides. On the burning of the organic substances the mineral bodies combined therewith are set free and may be eliminated. It is also admitted that they in part combine with the new products of the combustion, and in part with organic nutritive bodies poor in salts or nearly salt-free, which are absorbed from the intestinal canal and are thus retained (Voit, Forster [1]).

If this statement is correct, it is possible that a constant supply of mineral substances with the food is not absolutely necessary, and that the amount of inorganic bodies which must be administered is insignificant. The question whether this be so or not has not, especially in man, been sufficiently investigated; but generally we consider the need of mineral substances by man as very small. It may, however, be assumed that man usually takes with his food a considerable excess of mineral substances.

Experiments to determine the results of an insufficient supply of mineral substances with the food in animals have been made by several investigators, especially Forster. He observed, on experimenting with dogs and pigeons with food as poor as possible in mineral substances, that a very suggestive disturbance of the functions of the organs, particularly the muscles and the nervous system, appeared, and that death resulted in a short time, earlier in fact than in complete starvation. On observations made upon himself, Taylor [2] found on partaking less than 0.1 gram salts *per diem* that the chief disturbance occurred in the muscular system.

Bunge in opposition to these observations of Forster's has suggested that the early death in these cases was not caused by the lack of mineral salts, but more likely by the lack of bases necessary to neutralize the sulphuric acid formed in the combustion of the proteins in the organism; these bases must then be taken from the tissues. In accordance with this view, Bunge and Lunin [3] also found, in experimenting with mice, that animals which received nearly ash-free food with the addition of sodium carbonate were kept alive twice as long as those which had the same food without the sodium carbonate. Special experiments also

[1] Forster, Zeitschr. f. Biologie, 9. See also Voit in Hermann's Handbuch, 6, Part 1, 354. In regard to the occurrence and the behavior of the various mineral constituents of the animal body see the work of Albu and Neuberg, Physiologie und Pathologie des Mineralstoffwechsel, Berlin, 1906.

[2] University of California Publications, Pathol., 1.

[3] Bunge, Lehrbuch der physiol. Chem., 4. Aufl., 97; Lunin, Zeitschr. f. physiol. Chem., 5.

show that the carbonate cannot be replaced by an equivalent amount of sodium chloride, and that to all appearances it acts by combining with the acids formed in the body. The addition of alkali carbonate to the otherwise nearly ash-free food may indeed delay death, but cannot prevent it, and even in the presence of the necessary amount of bases death results from lack of mineral substances in the food.

With an insufficient supply of *chlorides* with the food the elimination of chlorine by the urine decreases constantly, and at last it may stop entirely, while the tissues still persistently retain the chlorides. It has already been stated (Chapter IX) how chloride starvation influences other functions, especially the secretion of gastric juice. If there be a lack of sodium as compared with potassium, or if there be an excess of potassium compounds in any other form than KCl, the potassium combinations are replaced in the organism by NaCl, so that new potassium and sodium compounds are produced which are voided with the urine. The organism becomes poorer in NaCl, which therefore must be taken in greater amounts from the outside (BUNGE). This occurs continuously in herbivora, and in man with vegetable food rich in potash. For human beings, and especially for the poorer classes of people who chiefly live on potatoes and foods rich in potash, common salt is, not only a condiment, but a necessary addition to the food (BUNGE [1]). On the behavior of chlorides, especially sodium chloride, in the animal body as well as the elimination or the retention of NaCl in diseases we have an abundance of investigations, which may be found in ALBU and NEUBERG'S work, previously cited.

Lack of Alkali Carbonates or Bases in the Food. The chemical processes in the organism are dependent upon the presence of tissue-fluids of a certain reaction, and this action, which is habitually alkaline toward litmus and neutral toward phenolphthalein, is chiefly due to the presence of alkali carbonates and carbon dioxide and in a lesser degree to alkali phosphates. The alkali carbonates are also of great importance, not only as a solvent for certain protein bodies and as constituents of certain secretions, such as the pancreatic and intestinal juices, but they are also a means of transportation of the carbon dioxide in the blood. It is therefore easy to understand that a decrease below a certain point in the quantity of alkali carbonate must endanger life. Such a decrease not only occurs with lack of bases in the food which brings about various disturbances and death by a relatively great production of acids through the burning of the proteins, but it also occurs when an animal is given dilute mineral acids for a period. The importance of ammonia as a means of neutralizing the acids produced or introduced into the body

[1] Zeitschr. f. Biologie, 9.

as well as the unequal resistance of man and other animals toward this action of acids has already been discussed in Chapter XV.

Lack of Phosphates and Earths. With the exception of the value of the alkaline earths as carbonates and more especially as phosphates in the physical composition of certain structures, such as the bones and teeth, their physiological importance is almost unknown. The importance of calcium for certain enzymotic processes and of calcium ions for the functions of the muscles, and especially for cell life, gives an indication of the necessity of the alkaline earths to the animal organism. Little is known of the need of these earths in adults, and no average results can be given. The same is true for the need of phosphates or phosphoric acid, whose value is recognized not only in the construction of the bones, but also in the functions of the muscles, the nervous system, the glands, the organs of generation, etc. The extent of this need is most difficult to determine, as the body shows a strong tendency, when increased amounts of phosphorus are introduced, to retain more than is necessary. The need of phosphates, which, according to EHRSTRÖM,[1] corresponds in adults to a minimum of 1 to 2 grams phosphorus, is relatively smaller in adults than in young, developing animals, and in these latter the question of the result of an insufficient supply of earthy phosphates and alkaline earths upon the bone tissue is of special interest. For details we refer to Chapter X and to the cited work of ALBU-NEUBERG.

Another important question is, How far do the phosphates take part in the construction of the phosphorized constituents of the body or to what extent are they necessary? The experiments of RÖHMANN and his pupils[2] with phosphorized (casein, vitellin) and non-phosphorized proteins (edestin) and phosphates show that with the introduction of casein and vitellin a deposition of nitrogen and phosphorus takes place, while with non-phosphorized protein and phosphates this does not seem to occur. The body apparently does not have the power of building up the phosphorized cell constituents necessary for cell life from non-phosphorized proteins and phosphates. On the contrary, according to the observations of several investigators, the lecithins seem to possess this power. As known from the investigations of MIESCHER, the development of generative organs of the salmon, which are very rich in nuclein substances and phosphatides, from the muscles which are relatively poor in organic-combined phosphorus, seem to indicate a synthesis of phosphorized organic substance from the phosphates. The recent investigations of HART, MACCOLLUM and FULLER,[3] who found that pigs with food poor in phosphorus develop just as well with inorganic phosphates as

[1] Skand. Arch. f. Physiol., **14**.

[2] See Marcuse, Pflüger's Arch., 67, and also footnote 2, page 826.

[3] Hart, MacCollum and Fuller, Amer. Journ. of Physiol., **23**.

with organic phosphorus compounds, also indicate such a formation. Other investigators, such as v. WENDT,[1] also admit of a synthesis of phosphorized protein substances by the aid of inorganic phosphates.

Lack of Iron. As iron is an integral constituent of hæmoglobin, absolutely necessary for the supply of oxygen, hence it is an indispensable constituent of food. Iron is a never-failing constituent of the nucleins and nucleoproteins, and herein lies another reason for the necessity of the introduction of iron. Iron is also of great importance in the action of certain enzymes, the oxidases. In iron starvation, iron is continually eliminated, even though in diminished amounts; and with an insufficient supply of iron with the food the formation of hæmoglobin decreases. The formation of hæmoglobin is not only enhanced by the supply of organic iron, but also, according to the general view, by inorganic iron preparations. The various divergent reports on this question have already been given in a previous chapter (on the blood).

In the absence of *protein bodies* in the food the organism must nourish itself by its own protein substances, and with such nutrition it must sooner or later succumb. By the exclusive administration of fat and carbohydrates the consumption of proteins in these cases is very considerably reduced. According to the doctrine of C. VOIT, which has been defended by recent investigations of E. VOIT and KÖRKUNOFF,[2] the protein metabolism is never so low under these conditions as in starvation. According to several investigators, such as HIRSCHFELD, KUMAGAWA, KLEMPERER, SIVÉN, LANDERGREN, and others, the protein metabolism may indeed, with exclusively fat and carbohydrate diet, be smaller than in complete starvation. Thus LANDERGREN has observed on an adult, healthy man in nitrogen starvation but with sufficient supply of energy (about 40 calories per kilo as carbohydrates and fat) on the fourth starvation day that the nitrogen excretion was not more than 4 grams. On the seventh day, with only carbohydrates, the nitrogen excretion sank to 3.34 grams, which corresponded to 0.047 gram N per kilo of body weight and to 0.29 gram protein. The recent investigations of MICHAÜD[3] on dogs also show that in protein starvation, as by exclusive feeding with fat and carbohydrate, lower results are obtained for the nitrogen elimination than in complete starvation.

The absence of *fats* and *carbohydrates* in the food affects carnivora and herbivora somewhat differently. It is not known whether carnivora can be kept alive for any length of time by food entirely free from fat

[1] Skand. Arch., f. Physiol., **17**.

[2] Zeitschr. f. Biologie, **32**.

[3] Hirschfeld, Virchow's Arch., **114**; Kumagawa, *ibid.*, **116**; Klemperer, Zeitschr. f. klin. Med., **16**; Sivén, Skand. Arch. f. Physiol., **10** and **11**; Landergren, l.c., **11**; footnote 3, page 837, also Maly's Jahresber., **32**; Michaud, Zeitschr. f. physiol. Chem., **59**.

and carbohydrates.[1] But it has been positively demonstrated that they can be kept alive a long time by feeding exclusively with meat freed as much as possible from visible fat (PFLÜGER[2]). Human beings and herbivora, on the contrary, cannot live for any length of time on such food. On one hand they lose the property of digesting and assimilating the necessarily large amounts of meat, and on the other a distaste for large quantities of meat or proteins soon appears.

A question of greater importance is whether it is possible to maintain life in an animal for any length of time with a mixture of simple organic and inorganic foodstuffs. This was not possible in the experiments of BUNGE and LUNIN, cited above. Later investigators, such as HALL and STEINITZ, FALTA and NOEGGERATH, KNAPP and JACOB have come to the same conclusion, although they obtained somewhat better results. RÖHMANN[3] obtained much better results, and he showed that the selection of the protein is of the greatest importance. "If mice are fed with a mixture of 7.2 parts vitellin, 14 parts casein, 4 egg albumin, 27 margarine, 120 wheat starch, 60 potato starch, 10 dextrose, 4 salts (a mixture of 10 parts calcium phosphate, 40 acid potassium phosphate, 20 sodium chloride, 15 sodium citrate, 8 magnesium citrate, 8 calcium lactate) it is not only possible to keep all the mice alive for some time but it is also possible to raise young mice by artificial feeding of the mother and then the small animals themselves (RÖHMANN[4]). They become mature and deliver active offspring who in turn become mature. The young that these deliver cannot be raised to maturity." If the food only contained one of the above-mentioned proteins the adult mice lost weight and succumbed after a certain time. If the casein is replaced by egg albumin or the egg albumin was replaced by casein the weight of all the mice remained constant for some time. Young mice had their development retarded in a remarkable manner. They remained at the same body weight for some time and then succumbed.

These experiments show that the various proteins have different importance in nutrition and especially for the develpoment of the young.

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[1] See Horbaczewski, Maly's Jahresber., **31**, 715.

[2] Pflüger's Arch., **50**.

[3] Hall, Arch. f. (Anat. u.) Physiol., 1896; Steinitz, Ueber Versuche mit künstlicher Ernährung, Inaug.-Diss., Breslau, 1900; Falta and Noeggerath, Hofmeister's Beiträge, **7**; Knapp, Zeitschr. f. exp. Path. u. Therap., **5**; Jacob, Zeitschr. f. Biol., **48**; Röhmann, Klin. therap. Wochenschr., No. 4, 1902 and Allg. med. Zentral.-Zeitg., 1908, No. 9.

[4] Allg. med. Zentral-Zeitg., 1908, No. 9.

III. METABOLISM WITH VARIOUS FOODS.

For carnivora, as above stated, meat as poor as possible in fat may be a complete and sufficient food. As the proteïns moreover take a special place among the organic nutritive bodies by the quantity of nitrogen they contain, it is proper that we first describe the metabolism with an exclusively meat diet.

Metabolism with food rich in proteins, or feeding only with meat as poor in fat as possible.

By an increased supply of proteins the catabolism and the elimination of nitrogen is increased, and this in proportion to the supply of proteins.

If a certain quantity of meat has daily been given to carnivora as food and the quantity is suddenly increased, an augmented catabolism of proteins, or an increase in the quantity of nitrogen eliminated, is the result. If the animal is daily fed for a certain time with larger quantities of the same meat, a part of the proteins accumulates in the body, but this part decreases from day to day, while there is a corresponding daily increase in the elimination of nitrogen. In this way a nitrogeneous equilibrium is established; that is, the total quantity of nitrogen eliminated is equal to the quantity of nitrogen in the absorbed proteins or meat. If, on the contrary, an animal which is in nitrogenous equilibrium, having been fed on large quantities of meat, is suddenly given a small quantity of meat per day, then it uses up its own body proteins, the amount decreasing from day to day. The elimination of nitrogen and the catabolism of proteins decrease constantly, and the animal may in this case also pass into nitrogeneous equilibrium, or almost into this condition. These relations are illustrated by the following table (Voit) [1]:

	Grams of Meat in the Food per Day.	
	Before the Test.	During the Test.
1.	500	1500
2.	1500	1000

Grams of Flesh Metabolized in Body per Day.

1	2	3	4	5	6	7
1222	1310	1390	1410	1440	1450	1500
1153	1086	1088	1080	1027		

In the first case (1) the metabolism of meat before the beginning of the actual experiment on feeding with 500 grams of meat was 447 grams, and it increased considerably on the first day of the experiment, after feeding with 1500 grams of meat. In the second case (2), in which the animal was previously in nitrogenous equilibrium with 1500 grams of meat, the metabolism of flesh on the first day of the experiment, with only 1000 grams meat, decreased considerably, and on the fifth day an almost

[1] Hermann's Handbuch, 6, Part I, 110.

nitrogeneous equilibrium was obtained. During this time the animal gave up daily some of its own proteins. Between that point below which the animal loses from its own weight and the maximum, which seems to be dependent upon the digestive and assimilative capacity of the intestinal canal, a carnivore may be kept in nitrogeneous equilibrium with varying quantities of proteins in the food.

The supply of proteins, as well as the protein condition of the body, affects the extent of the protein metabolism. A body which has become rich in proteins by a previous abundant meat diet must, to prevent a loss of proteins, take up more protein with the food than a body poor in proteins.

In regard to the rapidity with which the protein catabolism takes place FALTA[1] found in man but not, or at least not to the same extent, in dogs, that quite great differences exist between the different proteins. Thus on feeding pure proteins the chief amount of the nitrogen is more quickly eliminated after feeding casein than after genuine ovalbumin. This latter is more easily demolished after a previous modification by coagulation than in the native state, which indicates that an unequal resistance of the different proteins toward the digestive juices plays a part. HÄMÄLÄINEN and HELME[2] have also obtained similar results. Even on feeding with easily decomposable proteins it always takes several days before the total nitrogen corresponding thereto is eliminated, which depends, according to FALTA, upon a progressive demolition of the protein. From the unequal rate at which the different proteins are decomposed it follows that in the passage from a diet poor in protein to one rich in protein the time within which nitrogenous equilibrium occurs depends chiefly upon the kind of protein contained in the food.

PETTENKOFER and VOIT have made investigations on the *metabolism of fat* with an exclusively protein diet. These investigations have shown that by increasing the quantity of proteins in the food the daily metabolism of fat decreases, and they have drawn the conclusion from these experiments, as detailed in Chapter X, that there may even take place a formation of fat under these circumstances. The objections presented by PFLÜGER to these experiments, as well as the proofs of the formation of fat from proteins, are also given in the above-mentioned chapter.

According to PFLÜGER's doctrine, the protein can influence the formation of fat only in an indirect way, namely, in that it is consumed instead of the non-nitrogeneous bodies and hence the fat and fat-forming carbohydrates are spared. If sufficient protein is introduced with the food to satisfy the total nutritive requirements, then the decomposition of fat stops; and if non-nitrogenous food is taken at the same time, this is

[1] Deutsch. Arch. f. kiln. Med., 86.

[2] Skand. Arch. f. Physiol., 19.

not consumed, but is stored up in the animal body, the fats as such, and the carbohydrates at least in great part as fat.

PFLÜGER defines the "nutritive requirement" as the smallest quantity of lean meat which produces nitrogenous equilibrium without causing any decomposition of fat or carbohydrates. At rest and at an average temperature it is found in dogs to be 2.073 to 2.099 grams of nitrogen [1] (in meat fed) per kilo of flesh weight (not body weight, as the fat, which often forms a considerable fraction of the weight of the body, cannot as it were be used as dead measure). Even when the supply of protein is in excess of the nutritive requirements, PFLÜGER found that the protein metabolism increases with an increased supply until the limit of digestive power is reached, which limit is about 2600 grams of meat with a dog weighing 30 kilos. In these experiments of PFLÜGER'S not all of the excess of protein introduced was completely decomposed, but a part was retained by the body. PFLÜGER therefore defends the proposition "that a supply of proteins only, without fat or carbohydrate, does not exclude a protein fattening."

From what has been said on protein metabolism in starvation and with exclusive protein food it follows that the protein catabolism in the animal body never stops, that the extent is dependent in the first place upon the extent of protein supply, and that the animal body has the property, within wide limits, of accommodating the protein catabolism to the protein supply.

These and certain other peculiarities of protein catabolism have led VOIT to the view that not all proteins in the body are decomposed with the same ease. VOIT differentiates between the proteins fixed in the tissue-elements, so-called organized proteins, *tissue-proteins*, from those proteins which circulate with the fluids in the body and its tissues and which are taken up by the living cells of the tissues, from the interstitial fluids washing them, and destroyed. These *circulating proteins* are, he claims, more easily and quickly destroyed than the tissue-proteins. When, therefore, in a fasting animal which has been previously fed with meat, an abundant and quickly decreasing decomposition of proteins takes place, while in the further course of starvation this protein catabolism becomes less in quantity and more uniform, this depends upon the fact that the supply of circulating proteins is destroyed chiefly in the first days of starvation and the tissue-proteins in the last days.

The tissue-elements constitute an apparatus of a relatively stable nature, which has the power of taking proteins from the fluids washing the tissues and appropriating them, while their own proteins, the tissue-proteins, are ordinarily catabolized to only a small extent, about 1 per cent daily (VOIT). By an increased supply of proteins the activity of the cells and their ability to decompose nutritive proteins is also increased to a certain degree. When nitrogenous equilibrium is obtained after an

[1] See Schöndorff, Pflüger's Arch., 71.

increased supply of proteins, it indicates that the decomposing power of the cells for proteins has increased so that the same quantity of proteins is metabolized as is supplied to the body. If the protein metabolism is decreased by the simultaneous administration of other non-nitrogenous foods (see below), a part of the circulating proteins may have time to become fixed and organized by the tissues, and in this way the mass of the flesh of the body increases. During starvation or with a lack of proteins in the food the reverse takes place, for a part of the tissue proteins is converted into circulating proteins which are metabolized, and in this case the flesh of the body decreases.

Voit's theory has been criticised by several investigators and especially by Pflüger. Pflüger's belief, based on an investigation made by one of his pupils, Schöndorff,[1] is that the extent of protein destruction is not dependent upon the quantity of circulating proteins, but upon the nutritive condition of the cells for the time being—a view which does not widely differ from Voit if the author does not misunderstand Pflüger. Voit[2] has, as is known, stated that the conditions for the destruction of substances in the body exist in the cells, and also that the circulating protein is first catabolized after having been taken up by the cells from the fluids washing them. The point of Voit's theory is that all proteins are not destroyed in the body with the same degree of ease. The organized protein, which is fixed by the cells and has become a part of them, is destroyed less readily than the protein taken up by the cells from the nutritive fluid, which serves as material for the chemical construction of the much more complicated organized proteins. This nutritive protein, which circulates with the fluids before it is taken up by the cells, and which can exist in store in the cells as well as in the fluids, agreeably to Voit's view, has been called circulating protein or store protein by him. It is clear that these names may lead to misunderstanding, and therefore too much stress should not be put upon them. The most essential part of Voit's theory is the supposition that the food protein of the cells is more easily destroyed than the organized, real protoplasmic protein, and this assertion can hardly, for the present, be considered as refuted or exactly proven.

The investigations in recent years, especially those of Folin, which show that the amount of certain nitrogenous urinary constituents, such as creatinine, uric acid and the combinations containing neutral sulphur are almost independent of the quantity of protein taken as food, while the quantity of urea is determined by the protein partaken of, tend to substantiate Voit's view that we must differentiate between the real cell protein and the food protein. This has also led Folin to differen-

[1] Pflüger, Pflüger's Arch., **54**; Schöndorff, *ibid.*, **54**.

[2] Zeitschr. f. Biologie, **11**.

tiate between endogenous and exogenous protein metabolism. The experience on protein feeding and the endeavor of the body, as observed by SCHREUER,[1] on going to an ordinary diet after abundant protein feeding, to remain at the old state previous to the overfeeding of protein, point to the fact that protein retained by the body is not quite the same as the other body protein.

This question is intimately connected with another, namely, whether the food proteins taken up by the cells are metabolized as such or whether they are first organized, i.e., are converted into specific cell protein. The investigations of PANUM, FALCK, ASHER and HAAS and others[2] on the transitory progress of the elimination of nitrogen after a meal rich in proteins throws light on this question. From experiments upon a dog it was found that the elimination of nitrogen increases almost immediately after a meal rich in proteins, and that it reaches its maximum in about six hours, when about one-half of the quantity of nitrogen corresponding to the administered proteins is eliminated. If we also recollect that, according to an experiment of SCHMIDT-MÜLHEIM[3] upon a dog, about 37 per cent of the given proteins are absorbed in the first two hours after the meal and about 59 per cent in the course of the first six hours, it may then be inferred that the increased elimination of nitrogen after a meal is due to a catabolization of the digested and assimilated proteins of the food not previously organized. If it is admitted that the catabolized protein must have been organized, then the greatly increased elimination of nitrogen after a meal rich in proteins supposes a far more rapid and comprehensive destruction and reconstruction of the tissues than has been generally assumed.

The extensive cleavage of the proteins in digestion and the repeatedly observed deamidation of amino-acids in the animal body make it probable that the abundant elimination of nitrogen after a diet rich in proteins is in great part due to a progressive demolition of the food protein in digestion, whereby certain atomic complexes are more readily split than others. The abundant elimination of nitrogen by the urine after partaking of considerable protein may also depend in great part upon those nitrogenous atomic complexes which are split off and whose nitrogen is split off as ammonia and therefore cannot be used by the body. The abundant formation of ammonia in the cells of the digestive apparatus

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[1] Folin, Amer. Journ. of Physiol., **13**; Schreuer, Pflüger's Arch., **110**.

[2] Panum, Nord. Med. Arkiv., **6**; Falck, see Hermann's Handbuch, **6**, Part I, 107; Asher and Haas, Bioch. Zeitschr., **12**. For further information in regard to the curve of nitrogen elimination in man, see Tschenloff, Korrespond. Blatt Schweiz. Aerzte, 1896; Rosemann, Pflüger's Arch., **65**, and Veraguth, Journ. of Physiol., **21**; Schlosse, Maly's Jahresber., **31**.

[3] Arch. f. (Anat. u.) Physiol., 1879.

after food rich in proteins, as observed by NENCKI and ZALESKI,[1] seems to speak in favor of this view.

It has been stated above that other foods may decrease the catabolism of proteins. Gelatin is such a food. *Gelatin* and the *gelatin-formers* do not seem to be converted into protein in the body, and this last cannot be entirely replaced by gelatin in the food. For example, if a dog is fed on gelatin and fat, its body sustains a loss of proteins even when the quantity of gelatin is great enough so that the animal with an amount of fat and meat containing just the same quantity of nitrogen as the gelatin in question, remains in nitrogenous equilibrium. On the other hand, gelatin, as VOIT, PANUM, and OERUM[2] have shown, has great value as a means of sparing the proteins, and it may decrease the catabolism of proteins to a still greater extent than fats and carbohydrates. This is apparent from the following summary of VOIT's experiments upon a dog:

Food per Day.				Flesh.	
Meat	Gelatin.	Fat.	Sugar.	Catabolized.	On the Body.
400	0	200	0	450	−50
400	0	0	250	439	−39
400	200	0	0	356	+44

I. MUNK[3] has later arrived at similar results by means of more decisive experiments. He found that in dogs on a mixed diet which contained 3.7 grams protein per kilo of body, of which hardly 3.6 grams were catabolized, nearly five-sixths could be replaced by gelatin. The same dog catabolized on the second day of starvation three times as much protein as with the gelatin feeding. MUNK also states that gelatin has a much greater sparing action on proteins than the fats or the carbohydrates.

This ability of gelatin to spare the proteins is explained by VOIT by the fact that the gelatin is decomposed instead of a part of the circulating proteins, whereby a part of this last may be organized.

The recent investigations of KRUMMACHER and KIRCHMANN show the extent of the sparing action of gelatin upon proteins. The extent of protein destruction during gelatin feeding was compared with the extent of protein catabolism in starvation, and it was found that 35–37.5 per cent of the quantity of protein decomposed in starvation could be spared by gelatin. The physiological availability of gelatin was found by KRUMMACHER to be equal to 3.88 calories for 1 gram, which corresponds to about 72.4 per cent of the energy-content of the gelatin. KAUFMANN, who experimented upon dogs, found that one-fifth of the protein nitrogen could be readily replaced by gelatin nitrogen, while in an experiment upon

[1] Arch. des scienc. biol. de St. Pétersbourg, 4; Salaskin, Zeitschr. f. physiol. Chem., 25; Nencki and Zaleski, Arch. f. exp. Path. u. Pharm., 37.

[2] Voit, l. c., 123; Panum and Oerum. Nord. Med. Arkiv., 11.

[3] Pflüger's Arch., 58.

himself with 93 per cent gelatin nitrogen, 4 per cent tyrosine nitrogen, 2 per cent cystin nitrogen, and 1 per cent tryptophane nitrogen, he found instead of the equal quantity of protein nitrogen in the periods before and after, that the gelatin replaced by amino acids had almost the same physiological value as the proteins. The correctness of this action of the amino-acids has unfortunately not been confirmed by others. Rona and Michaelis,[1] who also proved that one-fifth of the protein metabolism could be replaced by gelatin, found that no mentionable increase in the the nutritive value of gelatin on the addition of tyrosin or tryptophane occurred.

The value of gelatin has been found by Murlin [2] to be dependent to a high degree upon the protein condition of the body, on the calorific value of the food and the quantity of carbohydrates in the latter. If in a man weighing 70 kilos, 51 calories per kilo were partaken, the quantity of nitrogen eliminated was 10 per cent more than the starvation value, and when two-thirds of the total calories partaken of were supplied by carbohydrates, 63 per cent of the total nitrogen could be replaced by gelatin nitrogen.

Gelatin may also somewhat decrease the consumption of fat, although it is of less value in this respect than the carbohydrates.

The nutritive value of proteoses (and peptones) stands in close relation to the nutritive value of the proteins and gelatin. The early investigations made by Maly, Plósz and Gyergyay, and Adamkiewicz have led to the conclusion that with food which contains no proteins besides peptones (proteoses) an animal may not only preserve its nitrogenous equilibrium, but its protein condition may even increase. According to recent and more exact investigations by Pollitzer, Zuntz, and Munk the proteoses have the same nutritive value as proteins, at least in short experiments. Pollitzer claims that this is true for different proteoses as well as for true peptone; but this does not agree with the experience of Ellinger,[3] who finds that the true antipeptone (gland peptone) is not able to replace proteins entirely or to prevent the loss of protein in the animal body. On the contrary, he claims it has, like gelatin, the property of sparing proteins. Voit long ago expressed a similar view. He believes that the proteoses and peptones may indeed replace the proteins for a short time, but not permanently;

[1] Krummacher, Zeitschr. f. Biologie, **42**; Kirchmann, *ibid.*, **40**; Kaufmann, Pflüger's Arch., **109**; Rona and Michaelis, Zeitschr. f. physiol. Chem., **50**.

[2] Amer. Journ. of Physiol., **19**.

[3] Maly, Pflüger's Arch., **9**; Plósz and Gyergyay, *ibid.*, **10**; Adamkiewicz, "Die Natur und der Nährwerth des Peptons" (Berlin, 1877); Pollitzer, Pflüger's Arch., **37**, 301; Zuntz, *ibid.*, **37**, 313; Munk, Centralbl. f. d. med. Wissensch., 1889, 20, and Deutsch. med. Wochenschr., 1889; Ellinger, Zeitschr. f. Biologie, **33** (literature).

they can spare the proteins, but cannot be converted into proteins. According to the researches of BLUM the different proteoses have various nutritive values. In his experiments the heteroproteose from fibrin could not replace the proteins of the food, while casein protoproteose had this property. In the researches of HENRIQUES and HANSEN [1] on white mice it was shown that the heteroproteose as well as the dysproteose (from WITTE peptone) had the ability of protecting the body from loss of nitrogen and that they deposited nitrogen. If the animal body has the ability of again constructing protein from simple fractions then there is nothing strange in these reports on the nutritive value of proteoses. The proteoses and peptones are formed by cleavages, and perhaps certain atomic complexes are absent which occur in the mixture of cleavage products and which are necessary for a regeneration of special protein bodies.

We have a number of investigations on the action of amides upon metabolism, which are mostly connected by the use of *asparagin*. These investigations have in part led to conflicting results; but they indicate that carnivora and herbivora act differently, that the results are dependent upon the rapidity with which the asparagin is absorbed and also upon the bacterial action in the intestine, and that in herbivora a protein-sparing action can be brought about by asparagin.[2] If, as is generally admitted, the amino-acids can serve in the building up of the proteins, then there is no use denying that their amides can also be used by the animal body. The most important facts in connection with the relation of the amino-acids to the protein metabolism and protein syntheses have been mentioned in a previous Chapter (IX).

Metabolism on a Diet consisting of Protein, with Fat or Carbohydrates. Fat cannot arrest or prevent the *catabolism of proteins;* but it can decrease it, and so spare the proteins. This is apparent from the following table of VOIT.[3] A is the average for three days, and B for six days.

	Food.		Flesh.	
	Meat.	Fat.	Metabolized.	On the Body.
A.	1500	0	1512	−12
B.	1500	150	1474	+26

[1] Blum, Zeitschr. f. physiol. Chem., **30**; Voit, l. c., 394; Henriques and Hansen, Zeitschr. f. physiol. Chem., **48**.

[2] Weiske, Zeitschr. f. Biologie, **15** and **17**, and Centralbl. f. d. med. Wissensch., 1890, 945; Munk, Virchow's Arch., **94** and **98**; Politis, Zeitschr. f. Biologie, **28**. See also Mauthner, *ibid.*, 28; Gabriel, *ibid.*, **29**; and Voit, *ibid.*, **29**, 125; Kellner, Maly's Jahresber., **27**, and Zeitschr. f. Biologie, **39**; Pflüger's Arch., **113**; Kellner and Köhler, Chem. Centralbl., **1**, 1906. Völtz, Pflüger's Arch., **107**, **117**, with Yakuwa, *ibid.*, **121**; v. Strusiewicz, Zeitschr. f. Biol., **47**; Rosenfeld and Lehmann, Pflüger's Arch., **112**; Lehmann, *ibid.*, **115**; M. Müller, *ibid.*, **117**; Henriques and Hansen, Zeitschr. f. physiol. Chem., **54**.

[3] Voit in Hermann's Handbuch, **6**, 130.

According to VOIT the adipose tissue of the body acts like the food-fat, and the protein-sparing effect of the former may be added to that of the latter, so that a body rich in fat may not only remain in nitrogenous equilibrium, but may even add to the store of body proteins, while in a lean body with the same food containing the same amount of proteins and fat there would be a loss of proteins. In a body rich in fat a greater quantity of proteins is protected from metabolism by a certain quantity of fat than in a lean body.

Because of the sparing action of fats an animal to whose food fat is added may, as is apparent from the table, increase its store of protein with a quantity of meat which is insufficient to preserve nitrogenous equilibrium.

Like the fats the carbohydrates have a sparing action on the proteins. By the addition of carbohydrates to the food carnivora not only remain in nitrogenous equilibrium, but the same quantity of meat which in itself is insufficient and which without carbohydrates would cause a loss of weight in the body may with the addition of carbohydrates produce a deposit of proteins. This is apparent from the following table.[1]

Food.				Flesh.	
Meat.	Fat.	Sugar.	Starch.	Metabolized.	On the Body.
500	250	...	...	558	− 58
500	...	300	...	466	+ 34
500	...	200	...	505	− 5
800	...	...	250	745	+ 55
800	200	...	...	773	+ 27
2000	...	...	200–300	1792	+208
2000	250	...	...	1883	+117

The sparing of protein by carbohydrates is greater, as shown by the table, than by fats. According to VOIT the first is on an average 9 per cent and the other 7 per cent of the administered protein without a previous addition of non-nitrogenous bodies. Increasing quantities of carbohydrates in the food decrease the protein metabolism more regularly and constantly than increasing quantities of fat. ATWATER and BENEDICT[2] also found that the carbohydrates had a somewhat greater sparing action upon proteins than fats.

Because of this great protein-sparing action of carbohydrates the herbivora, which as a rule partake of considerable quantities of carbohydrates, assimilate proteins readily (VOIT).

The greater protein-sparing action of carbohydrates as compared with that of the fats occurs, as shown by LANDERGREN,[3] to a still higher degree with food poor in nitrogen or in nitrogen starvation, in which cases the

[1] Voit, *ibid.*, page 143.

[2] See Ergebnisse der Physiologie, **3**.

[3] l. c., Inaug.-Diss., and Skand. Arch. f. Physiol., **14**.

carbohydrates have double the protein-sparing action as compared with an isodynamic quantity of fat.

The protein-sparing action of the carbohydrates and fats has generally been studied through the one-sided feeding with one or the other of these two groups of foodstuffs. The question may be raised whether the difference observed between the fats and carbohydrates could not also be brought about by the simultaneous supply of carbohydrates and fat in varying proportions. TALLQUIST [1] made a series of experiments on this subject. In one of the periods 16.27 grams N, 44 grams fat, and 466 grams carbohydrate were given; in a second, 16.08 grams N, 140 grams fat, and 250 grams carbohydrate, containing almost the same number of calories, namely, 2867 and 2873. In both cases an almost complete nitrogenous equilibrium was reached and the carbohydrate did not spare more protein than the fat. It is therefore possible that the fat has about the same protein-sparing action as an isodynamic amount of carbohydrate when the quantity of carbohydrates does not sink below a certain minimum, which is not known for the present.

This condition as well as the extent of protein-sparing action of the carbohydrates stands, according to LANDERGREN,[2] in close relation to the formation of sugar in the body. The animal body always needs sugar, and a lack of carbohydrates in the food leads to a part of the proteins being used in the sugar formation. This part can be spared by carbohydrates but not by fats, from which, according to LANDERGREN, the carbohydrates cannot be formed. In this also lies the probable reason why the fats, on being fed exclusively but not with a sufficient supply of carbohydrates, have a much lower protein-sparing action than the carbohydrates. The fats cannot prevent the protein catabolism necessary for the formation of sugar on a diet lacking in carbohydrates.

The law as to the increased protein catabolism with increased protein supply also applies to food consisting of protein with fat and carbohydrates. In these cases the body tries to adapt its protein catabolism to the supply; and when the daily calorie-supply is completely covered by the food, the organism can, within wide limits, be in nitrogenous equilibrium with different quantities of protein.

The upper limit to the possible protein catabolism per kilo and per day has been determined only for herbivora. For human beings it is not known, and its determination is from a practical standpoint of secondary importance. What is more important is to ascertain the lower limit, and on this subject we have several experiments upon man as well as upon

[1] Finska Lakaresällskapets Handl., 1901. See also Arch. f. Hygiene, **41**.

[2] l. c., Inaug.-Diss. See also Skand. Arch. f. Physiol., **14**.

dogs by HIRSCHFELD, KUMAGAWA, KLEMPERER, MUNK, ROSENHEIM,[1] and others. It follows from these experiments that the lower limit of protein needed for human beings, for a week or less, is about 30–40 grams or 0.4–0.6 gram per kilo with a body of average weight. v. NOORDEN[2] considers 0.6 gram protein (absorbed protein) per kilo and per day as the lower limit. The above-mentioned figures are valid only for short series of experiments; still there exists the observation of E. VOIT and CONSTANTINIDI[3] on the diet of a vegetarian when the protein condition was kept almost but not completely normal for a long time with about 0.6 gram of protein per kilo. CASPARI[4] has also made observations upon a vegetarian for a period of 14 days with an average of 0.1 gram nitrogen (recalculated as equal to 0.62 gram protein) per kilo, where a nearly complete nitrogenous equilibrium was observed as the average result.

According to VOIT's normal figures, which will be spoken of below, for the nutritive need of man, an average workingman of about 70 kilos weight, requires on a mixed diet about 40 calories per kilo (true calories or net calories). In the above experiments with food very poor in protein the demand for calories was considerably greater; as, for instance, in certain cases it was 51 (KUMAGAWA) or even 78.5 calories (KLEMPERER). It therefore seems as if the above very low supply of protein was possible only with great waste of non-nitrogenous food; but in opposition to this it must be recalled that in VOIT and CONSTANTINIDI's experiments upon the vegetarian, who for years was accustomed to a food poor in protein and rich in carbohydrate, the calories amounted to only 43.7 per kilo. In the case studied by CASPARI a supply of 41 calories per kilo was entirely sufficient.

SIVÉN has shown by experiments upon himself that the adult human organism, at least for a short time, can be maintained in nitrogenous equilibrium with a specially low supply of nitrogen without increasing the calories in the food above the normal. With a supply of 41-43 calories per kilo he remained in nitrogenous equilibrium for four days with a supply of nitrogen of 0.08 gram per kilo of body weight. Of the nitrogen taken, a part was of a non-protein nature and the quantity of true protein nitrogen was only 0.045 gram, corresponding to about 0.3 gram of protein per kilo of body weight. That this low limit, which by the way holds only for a short time, has no general validity follows from other observations. Thus CASPARI[5] also, in an experiment on himself, could not attain com-

[1] See footnote 3, page 846; also Munk, Arch. f. (Anat. u.) Physiol., 1891 and 1896; Rosenheim, *ibid.*, 1891; Pflüger's Arch., **54**.

[2] Grundriss einer Methodik der Stoffwechseluntersuchungen. Berlin, 1892.

[3] Zeitschr. f. Biologe, **25**.

[4] Physiologische Studien über Vegetarismus, Bonn, 1905.

[5] Sivén, Skand. Arch. f. Physiol., **10** and **11**; Caspari, Arch. f. (Anat. u.) Physiol., 1901.

plete nitrogenous equilibrium on a much greater nitrogen supply. The protein minimum also seems to vary in different individuals.

The protein minimum can also be different for other reasons. It varies, as mentioned by RUBNER, not only with the kind of foodstuffs, but also with the nutritive condition of the body. The needs of the cells for protein varies with the nutritive condition of the body. Where the protein is eagerly demanded, less supply of protein suffices, and where the demand is low more protein must be offered (RUBNER). The more the body has become reduced the lower is the protein minimum, according to RUBNER.[1]

As mentioned in the early part of this chapter, the body always suffers a certain loss of nitrogen through the falling out of the hair and other epidermis formations, by the secretions, etc.; but to this also belongs the constant loss of nitrogenous substance which every cell sustains because of its activity. This unpreventable loss of nitrogen has been included by RUBNER under the name "wear and tear" quota, and this quota, which corresponds to the nitrogen elimination with a perfectly nitrogen-free diet, and hence is a protein minimum, may rise to 4 per cent of the total calorific needs. The energy supply of the food is under these conditions entirely assumed by the non-nitrogenous foodstuffs.

All proteins do not have the same value in replacing the protein minimum. MICHAUD[2] determined the protein minimum in dogs by feeding entirely with nitrogen-free food, and he found that this minimum can be covered by the corresponding quantity of protein specific of the animal, but not by the same quantity of a foreign protein, like gliadin and edestin.

The purposes of the protein as foodstuff are, according to RUBNER, as follows: (1) To compensate for the wear and tear quota; (2) betterment of the condition of the cells; and (3) dynamogenic purpose. In the accomplishment of this third purpose the protein splits into a nitrogenous and a non-nitrogenous part. The potential energy set free immediately as heat in the combustion of the nitrogenous part, which is quantitatively used within the region of the chemical heat regulation but is otherwise lost, has been called the *specific dynamic action* by RUBNER.[3] The remainder of the energy which is represented by the non-nitrogenous part of the proteins, serves, like all other foodstuffs, in satisfying the energy requirement of the cells. According to RUBNER only non-nitrogenous groups (of the proteins, fats and carbohydrates) come almost entirely, if not completely, in consideration for purposes of energy.

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[1] Rubner, Theorie d. Ernährung nach Vollendung des Wachstums, Arch. f. Hyg., 66. 1–80 and Ernährungsvorgänge beim Wachstum des Kindes, *ibid.*, 66, 81–126.

[2] Zeitschr. f. physiol. Chem., 59.

[3] Rubner, l. c., and Gesetze des Energieverbrauches, 70.

In close relation to the second purpose, the betterment of the condition of the cells, stands the question as to the conditions favoring the deposition of flesh in the body, which is closely associated with the question as to the conditions of fattening the body. In this connection it must be remembered in the first place that all fattening presupposes an overfeeding, i.e., a supply of foodstuffs which is greater than that catabolized in the same time.

In carnivora a flesh deposition may take place on the exclusive feeding with meat. This is not generally large in porportion to the quantity of protein catabolized. In man and herbivora, who cannot cover their calorific needs by protein alone, this is not possible, and the question as to the conditions of fattening with a mixed diet is of importance.

These conditions have also been studied in carnivora, and here, as Voit has shown, the relation between protein and fat (and carbohydrates) is of great importance. If much fat is given in proportion to the protein of the food, as with average quantities of meat with considerable addition of fat, then nitrogenous equilibrium is but slowly attained and the daily deposit of flesh, though not large, is quite constant, and may become greater in the course of time. If, on the contrary, much meat besides proportionately little fat is given, then the deposit of protein with increased catabolism is smaller day by day, and nitrogenous equilibrium is attained in a few days. In spite of the somewhat larger deposit *per diem*, the total flesh deposit is not considerable in these cases. The following experiment of Voit may serve as example:

Number of Days of Experimentation.	Food.		Total Deposit of Flesh.	Daily Deposit of Flesh.	Nitrogenous Equilibrium.
	Meat, Grams.	Fat, Grams.			
32	500	250	1792	56	Not attained
7	1800	250	854	122	Attained

The greatest absolute deposition of flesh in the body was obtained in these cases with only 500 grams of meat and 250 grams of fat, and even after 32 days nitrogenous equilibrium had not occurred. On feeding with 1800 grams of meat and 250 grams of fat nitrogenous equilibrium was established after seven days; and though the deposition of flesh per day was greater, still the absolute deposit was not one-half as great as in the former case.

The possibility of a protein fattening in man and animals (dogs, sheep) are shown by the series of experiments of Krug, Bornstein, Schreuer, Henneberg, Pfeiffer and Kalb,[1] and there is no doubt that such a

[1] Krug, Cited by v. Noorden, Lerb. der Path. des Stoffwechsel, 1. Aufl., p. 120; Bornstein, Berl. klin. Wochenschr., 1898, and Pflüger's Arch., **83** and **106**; Bornstein

fattening is possible. That we are here not dealing with an increase in the number of cells, but rather an enlargement of the volume of the same is the generally accepted view. Different theories as to the value of this protein fattening are entertained. According to a prevailing opinion this enrichment of protein is not continuous, but may easily be retrograde, and the question whether we are here dealing chiefly with a taking up of proteins by the cells (LÜTHJE) or with a new formation of living tissues, requires further solution.

It is difficult to produce a permanent flesh deposit in adult man by overfeeding alone. It is to a much greater degree a function of the specific growth energy of the cells and the cell-work than the excess of food. Therefore there is observed, according to v. NOORDEN, abundant flesh deposition (1) in each growing body; (2) in those no longer growing but whose body is accustomed to increased work; (3) whenever, by previous insufficient food or by disease, the flesh condition of the body has been diminished and therefore requires abundant food to replace it. The deposition of flesh is in this case an expression of the regenerative energy of the cells.[1]

The experiences of graziers show that in food-animals a flesh deposit does not occur, or at least is only inconsiderable, on overfeeding. The individuality and the race of the animal are of importance for flesh deposition.

The conditions in young, growing individuals are different than in adults. In the first the protein is necessary for the building up of the growing tissue and in them an abundant true flesh deposition takes place. For this protein fattening the amount of supply does not take first place, but rather the energy of development.

As above stated (Chapter X), respecting the formation of fat in the animal body, the most essential condition for a fat deposition is an overfeeding with non-nitrogenous foods. The extent of fat deposition is determined by the excess of calories administered over those actually needed. But as protein and fat are expensive nutritive bodies as compared with carbohydrates, the supply of greater quantities of carbohydrates is important for fat deposition. The body decomposes less substances at rest than during activity. Bodily rest, besides a proper combination of the three chief groups of organic foods, is therefore also an essential requisite for an abundant fat deposit.

Action of Certain Other Bodies on Metabolism. *Water.* If a quantity in excess of that which is necessary, is introduced into the organism, the excess is quickly and principally eliminated with the urine. This

and Schreuer, Pflüger's Arch., **110**; Henneberg and Pfeiffer, see Maly's Jahresb., **20**; Pfeiffer and Kalb, *ibid.*, **22**.

[1] See also Svenson, Zeitschr. f. klin. Med., **43**.

increased elimination of urine causes in fasting animals (VOIT, FORSTER), but not to any appreciable degree in animals taking food (SEEGEN, SALKOWSKI and MUNK, MAYER, DUBELIR [1]), an increased elimination of nitrogen. The reason for this increased nitrogen excretion is to be found in the fact that the drinking of much water causes a complete washing out of the urea from the tissues. Another view, which is defended by VOIT, is that because of the more active current of fluids, after taking large quantities of water, an increased metabolism of proteins takes place. VOIT considers this explanation the correct one, although he does not deny that by the liberal administration of water a more complete washing out of the urea from the tissues takes place. Opinions on this subject are not yet in accord, and recently HEILNER [2] has advocated VOIT'S view. The introduction of water in a starving animal is, HEILNER believes, to a certain extent an excessive supply of a foodstuff; hence the metabolism is increased and the water acts under these conditions in a sort of specific dynamic manner.

In this connection it must be mentioned that, according to HEILNER,[3] urea, when introduced subcutaneously in physiological salt solution, causes an increased action upon the protein catabolism, a fact which he mentions as an example that the products of metabolism probably have an influence upon the same processes which produce them.

When the body has lost a certain amount of water, then the abstinence from water (in animals) is accompanied by a rise in the protein metabolism (LANDAUER, STRAUB [4]). In regard to the action of water on the formation of fat and its metabolism, the theory that the free drinking of water is favorable for the deposition of fat seems to be generally admitted, while the drinking of only very little water acts against its formation. For the present we have no conclusive proofs of the correctness of this view.

Salts. In regard to the action of salts—for example sodium chloride and the neutral salts—which partly depends upon the use of large and varying amounts of salt in the experiments the authors disagree. Investigations of STRAUB and ROST [5] show that the action of salts stands in close relation to their power of abstracting water. Small amounts of

[1] Voit. Untersuch. über den Einfluss des Kochsalzes, etc. (München, 1860); Forster, cited from Voit in Hermann's Handbuch, **6**, 153; Seegen, Wien. Sitzungsber., **63**; Salkowski and Munk, Virchow's Arch., **71**; Mayer, Zeitschr. f. klin. Med., **2**; Dubelir, Zeitschr. f. Biologie, **28**.

[2] See R. Neumann, Arch. f. Hygiene, **36**; Heilner, Zeitschr. f. Biologie, **47** and **49**; Hawk, University of Pennsylvania Med. Bull., xviii.

[3] Zeitschr. f. Biol., **52**.

[4] Landauer, Maly's Jahresber., **24**; Straub, Zeitschr. f. Biologie, **37**.

[5] W. Straub, Zeitschr. f. Biologie, **37** and **38**; Rost, Arbeiten aus d. Kaiserliche Gesundheitsamte, **18** (literature). See also Grüber, Maly's Jahresber., **30**, 612.

salt which do not produce diuresis have no action on metabolism. On the contrary, larger amounts, which bring about a diuresis, which is not compensated by the ingestion of water, produce a rise in the protein metabolism. If the diuresis is compensated by drinking water, then the protein metabolism is not increased by salts, but is diminished to a slight degree. An increased nitrogen excretion caused by taking salts can be increased by the ingestion of water, thus increasing the diuresis, and the action of salts seems to bear a close relation to the demand and supply of water.

Alcohol. The question as to how far the alcohol absorbed in the intestinal canal is burnt in the body, or whether it leaves the body unchanged by various channels, has been the subject of much discussion. To all appearances the greatest part of the alcohol introduced (95 per cent or more) is burnt in the body (STUBBOTIN, THUDICHUM, BODLÄNDER, BENEDICENTI [1]). As the alcohol has a high calorific value (1 gram = 7 calories), then the question arises whether it acts sparingly on other bodies, and whether it is to be considered as a nutritive substance. The earlier investigations made to decide these questions have led to no decisive result. The thorough investigations of ATWATER and BENEDICT, ZUNTZ and GEPPERT, BJERRE, CLOPATT, NEUMANN, OFFER, ROSEMANN,[2] and others, seem to show positively that, in man, alcohol can diminish the consumption not only of fat and carbohydrates, but also the proteins, although at first, due to its poisonous properties, it may increase the protein metabolism for a short time. The nutritive value of alcohol can be of special importance in certain cases only, as large amounts of alcohol taken at one time, or the continued use of smaller quantities, has an injurious action on the organism. Alcohol may therefore be regarded as a foodstuff only in exceptional cases, and in other respects must be considered as an article of luxury.

Coffee and *tea* have no action on the exchange of material which can be positively proven, and their importance lies chiefly in their action upon the nervous system. It is impossible to enter into the effect of various therapeutic agents upon metabolism.

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[1] Arch. f. (Anat. u.) Physiol., 1896, which contains the literature.

[2] In regard to the literature on this subject, see the works of O. Neumann, Arch. f. Hygiene, **36** and **41**, and Rosemann, Pflüger's Arch., **86** and **94**. A summary of the entire literature upon alcohol can be found in Abderhalden, "Bibliographie der gesamten wissenschaftlichen Literature über den Alcohol und den Alcoholismus," Berlin and Wien, 1904. See also Rosemann in Oppenheimer's Handb. d. Bioch., Bd., 4. 1.

IV. THE DEPENDENCE OF METABOLISM ON OTHER CONDITIONS.

The so-called basal requirement which was previously mentioned, i.e., the extent of metabolism with absolute rest of body and inactivity of the intestinal tract, serves best as a starting-point for the study of metabolism under various external circumstances. The metabolism going on under these conditions leads in the first place to the production of heat, and it is only to a subordinate degree dependent upon the work of the circulatory and respiratory apparatus and the activity of the glands. According to a calculation by ZUNTZ,[1] only 10–20 per cent of the total calories of the basal requirement belongs to the circulation and respiration work.

The magnitude of the basal requirement depends in the first place upon the heat production necessary to cover the loss of heat, and this heat production is in turn dependent upon the relation between the weight and the surface of the body.

Weight of Body and Age. The greater the mass of the body the greater the absolute consumption of material; while, on the contrary, other things being equal, a small individual of the same species of animal metabolizes absolutely less, but relatively more as compared with the unit of the weight of the body. With increasing bodily weight the total metabolism per kilo of animal diminishes, which is true first for individuals of the same species of animals, but also seems to have a certain correctness on the comparison of different species of animals. It must be remarked that the relation between flesh and fat in the body exerts an important influence. The extent of the metabolism is dependent upon the quantity of active cells, and a very fat individual therefore decomposes less substance per kilo than a lean person of the same weight. According to RUBNER[2] the importance of the size of the flesh or cell-mass in the body is overestimated. In his investigations on two boys, one of whom was corpulent and the other normally developed, and on comparing the food-need with that found by CAMERER for boys of the same weight, RUBNER came to the result that the exchange of force in the corpulent boy almost completely corresponded with that in the non-corpulent boy of the same weight. By approximately estimating the quantity of fat in the body RUBNER was also able, from the protein condition, to compare the calculated exchange of energy with that actually found. The exchange per kilo amounted to 52 calories in the lean and 43.6 calories in the fat boy, while, if the protein condition was a measure, one would expect an exchange of calories of only 35 calories for the fat person. We cannot

[1] Cited from v. Noorden's Handbuch. 2. Aufl, page 97.

[2] Beitrage zur Ernährung im Knabenalter, etc. Berlin, 1902.

therefore admit of a diminished activity of the cell-mass in the fat boy, but rather an increased activity. According to RUBNER it is not the flesh-mass (protein mass) alone, but its variable functional changes, which determines the extent of decomposition. In women, who generally have less body weight and a greater quantity of fat than men, the metabolism in general is smaller, and the latter is ordinarily about four-fifths that of men.

The essential reason why small animals catabolize relatively more substance than large ones, when calculated per kilo body weight, is that the bodies of smaller animals have greater surface in proportion to their mass. On this account the loss of heat is greater, which causes increased heat production, i.e., a more active metabolism. This is also the reason why young individuals of the same kind show a relatively greater metabolism than older ones. If the heat production and carbon-dioxide elimination is calculated on the unit of surface of the body, we find, on the contrary, as the experiments of RUBNER, RICHET,[1] and others show, that they vary only slightly from a certain average in individuals of different weights.

According to RUBNER'S rule as to the influence of the surface, which has been recently formulated by E. VOIT, the need of energy in homoiothermic animals is influenced by the development of their surface when their body is given rest, medium surrounding temperature, and relatively equal protein condition. This rule applies not only to adult human beings, but also to children and growing individuals (RUBNER, OPPENHEIMER). The surface is the essential factor in determining the extent of exchange of energy. In order to show this we will give here, from a work of RUBNER,[2] the figures representing the quantity of heat in calories for 1 square meter of surface for twenty-four hours:

Adult, medium diet, rest	1189 calories.
Adult, medium diet, work	1399 "
Suckling	1221 "
Child with medium diet	1447 "
Aged men and women	1099 "
Women	1004 "

The variation in the calorific values[3] found by many investigators, which is sometimes not very small, suggests the fact that the surface rule is not alone decisive for the exchange of material in resting animals. Still it is generally considered that it is of the greatest importance in metabolism.

The more active metabolism in young individuals is apparent when

[1] Rubner, Zeitschr. f. Biologie, **19** and **21**; Richet, Arch. de Physiol., 5., (2).

[2] Rubner, Ernährung im Knabenalter, page 45; E. Voit, Zeitschr. f. Biologie, **41**; Oppenheimer, *ibid.*, **42**.

[3] See Magnus-Levy, Pflüger's Arch., **55**; Slowtzoff (u. Zuntz), *ibid.*, **95**.

we measure the gaseous exchange as well as the excretion of nitrogen. As example of the elimination of urea in children the following results of CAMERER [1] are of value:

Age.	Weight of Body in Kilos.	Urea in Grams. Per Day.	Per Kilo.
1½ years	10.80	12.10	1.35
3 "	13.30	11.10	0.90
5 "	16.20	12.37	0.76
7 "	18.80	14.05	0.75
9 "	25.10	17.27	0.69
12½ "	32.60	17.79	0.54
15 "	35.70	17.78	0.50

In adults weighing about 70 kilos, from 30 to 35 grams of urea per day are eliminated, or 0.5 gram per kilo. At about fifteen years of age the destruction of proteins per kilo is about the same as in adults. The relatively greater metabolism of proteins in young individuals is explained partly by the fact that the metabolism of material in general is more active in young animals, and partly by the fact that young animals are, as a rule, poorer in fat than those full grown.

That young individuals show a more active metabolism than adults, follows, as above stated, principally from the relatively greater body surface in the first as compared to the latter. According to TIGERSTEDT and SONDÉN, the greater metabolism in young animals depends nevertheless, also in part, on the fact that in these individuals the decomposition in itself is more active than in older ones. The period of growth has a considerable influence on the extent of metabolism (in man), and indeed the metabolism, even when calculated on the unit of surface of body, is greater in youth than in old age. This view is strongly disputed by RUBNER. He does not deny that differences exist between young and adult individuals which may be considered as a deviation from the above rule; still these differences may, he claims, be dependent upon the work performed, the food, and the nutritive condition. MAGNUS-LEVY and FALK [2] have reported observations which support the conclusions of SONDÉN and TIGERSTEDT.

Nurslings have a behavior different from older children, as with them during the first months of life, and especially the first three days, the metabolism, calculated on the unit of surface, is strikingly low, and lower than with adults. After about two weeks it attains about the same height as adults (SCHERER, FORSTER [3]).

In old age the metabolism is very much reduced; and even when calcu-

[1] Zeitschr. f. Biologie, **16** and **20**.

[2] Tigerstedt and Sondén, l. c.; Rubner, l. c.; and Arch. f. Hygiene, **66**; Magnus-Levy, Arch. f. (Anat. u.) Physiol., 1899, Suppl.

[3] Cited by A. Loewy in Oppenheimer's Handb., Bd. **4**, 189.

lated upon the square meter of surface of body it is lower than in an individual of medium age.

The question as to what extent *sex* specially influences metabolism remains to be investigated. TIGERSTEDT and SONDÉN found that in the young the carbon-dioxide elimination, per kilo of body weight as well as per square meter of body surface, was considerably greater in males than in females of the same age and the same weight of body. This difference between the two sexes seems to disappear gradually, and at old age it is entirely absent. The investigations of MAGNUS-LEVY and FALK oppose these observations. They investigated by means of the ZUNTZ-GEPPERT method, not only children, but also adults and old persons of both sexes, but could not observe any positive influence of the sex upon metabolism.[1]

As the metabolism may be kept at its lowest point by absolute rest of body and inactivity of the intestinal tract, it is manifest that work and the ingestion of food have an important bearing on the extent of metabolism.

Rest and Work. During work a greater quantity of chemical energy is converted into kinetic energy, i.e., the metabolism is increased more or less on account of work.

As explained in a previous chapter (XI), work, according to the generally accepted view, has no material influence on the excretion of nitrogen. It is nevertheless true that several investigators have observed, in certain cases, an increased elimination of nitrogen; this increase does not seem to be directly related to the work, but to be caused by secondary circumstances. These observations have been explained in other ways. For instance, work may, when it is connected with violent movements of the body, easily cause dyspnœa, and this last, as FRÄNKEL[2] has shown, may occasion an increase in the elimination of nitrogen, since diminution of the oxygen supply increases the protein metabolism. In other series of experiments the quantity of carbohydrates and fats in the food was not sufficient; the supply of fat in the body was decreased thereby, and the destruction of proteins was correspondingly increased. Other conditions, such as the external temperature and the weather,[3] thirst, and drinking of water, can also influence the excretion of nitrogen. The

[1] Tigerstedt and Sondén, Skand. Arch. f. Physiol., 6; Magnus-Levy and Falk, Arch. f. (Anat. u.) Physiol., 1899, Suppl. In regard to metabolism and its relation to the phases of sexual life and especially under the influence of menstruation and pregnancy, see the investigations of A. Ver Eecke (Bull. acad. roy. de méd. de Belgique, 1897 and 1901, and Maly's Jahresber., 30 and 31). See also Magnus-Levy in v. Noorden's Handb. d. Pathol. d. Stoffwechsels.

[2] Virchow's Arch., 67 and 71. In regard to disputed views see C. Voit, Zeitschr. f. Biol., 49 and Frankel, *ibid.*, 50.

[3] See Zuntz and Schumburg, Arch. f. (Anat. u.) Physiol., 1895.

prevailing sentiment is that muscular activity has hardly any influence on the metabolism of proteins.

On the contrary, work has a very considerable influence on the elimination of carbon dioxide and the consumption of oxygen. This action, which was first observed by LAVOISIER, has later been confirmed by many investigators. PETTENKOFER and VOIT[1] have made investigations on a full-grown man as to the metabolism of the nitrogenous as well as of the non-nitrogenous bodies during rest and work, partly while fasting and partly on a mixed diet. The experiments were made on a full-grown man weighing 70 kilos. The results are contained in the following table:

		Consumption of				
		Proteins.	Fat.	Carbohydrates.	CO_2 Eliminated.	O Consumed.
Fasting....	Rest.....	79	209	...	716	761
	Work.....	75	380	...	1187	1071
Mixed diet.	Rest.....	137	72	352	912	831
	Work.....	137	173	352	1209	980

In these cases work did not seem to have any influence on the destruction of proteins, while the gas exchange was considerably increased.

ZUNTZ and his pupils[2] have made important investigations into the extent of the exchange of gas as a measure of metabolism during work and caused by work. These investigations not only show the important influence of muscular work on the catabolism of material, but they also indicate, in a very instructive way, the relation between the extent of metabolism of material and its utilization for work of various kinds. We can refer only to those which are of special physiological interest.

The action of muscular work on the gas exchange does not alone appear with hard work. From the researches of SPECK and others we learn that even very small, apparently quite unessential movements may increase the production of carbon dioxide to such an extent that by not observing these, as in numerous older experiments, very considerable errors may creep in. JOHANSSON[3] has also made experiments upon himself, and finds that on the production of as complete a muscular inactivity as possible the ordinary amount of carbon dioxide (31.2 grams per hour at rest in the ordinary sense) may be reduced nearly one-third, or to an average of 22 grams per hour.

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[1] Zeitschr. f. Biologie, 2.

[2] See the works of Zuntz and Lehmann, Maly's Jahresber., 19; Katzenstein, Pflüger's Arch., 49; Lœwy, *ibid.*; Zuntz, *ibid.*, 68, Zuntz and Slowtzoff, *ibid.*, 95; and especially the large work "Untersuch. über den Stoffwechsel des Pferdes bei Ruhe und Arbeit," Zuntz and Hagemann, Berlin, 1898; Hohenklima und Bergwanderungen by Zuntz, Loewy, Müller and Caspari, which also contains a bibliography.

[3] Nord. Med. Arkiv. Festband, 1897; also Maly's Jahresber., 27; Speck, "Physiol. des menschl. Atmens," Leipzig, 1892.

The quantity of carbon dioxide eliminated during a working period is uniformly greater than the quantity of oxygen taken up at the same time, and hence a raising of the respiratory quotient was usually considered as caused by work. This rise does not seem to be based upon the character of chemical processes going on during work, as we have a series of experiments made by ZUNTZ and his collaborators, LEHMANN, KATZENSTEIN and HAGEMANN,[1] in which the respiratory quotient remained almost wholly unchanged in spite of work. According to LOEWY[2] the combustion processes in the animal body go on in the same way in work as in rest, and a raising of the respiratory quotient (irrespective of the transient change in the respiratory mechanism) takes place only with insufficient supply of oxygen to the muscles, as in continuous fatiguing work or excessive muscular activity for a brief period, also with local lack of oxygen caused by excessive work of certain groups of muscles. This varying condition of the respiratory quotient has been explained by KATZENSTEIN by the statement that during work two kinds of chemical processes act side by side. The one depends upon the work which is connected with the production of carbon dioxide also in the absence of free oxygen, while the other brings about the regeneration which takes place by the taking up of oxygen. When these two chief kinds of chemical processes make the same progress the respiratory quotient remains unchanged during work; if by hard work the decomposition is increased as compared with the regeneration, then a raising of the respiratory quotient takes place. If, on the contrary, moderate work is continued and performed in a way so that irregularities and occasional changes in the circulation and respiration are excluded or are without importance, then the respiratory quotient may correspondingly remain the same during work as in rest. Its extent is thus determined in the first place by the nutritive material at its disposal (ZUNTZ and his pupils).

The theory of LOEWY and ZUNTZ, that the raising of the respiratory quotient during work is to be explained by an insufficient supply of oxygen, is opposed by LAULANIÉ.[3] He has observed the reverse, namely, a diminution in the respiratory quotient during continuous excessive work, and this is not reconcilable with the above statements. He considers that sugar is the source of muscular energy, and that the rise in the respiratory quotient is due to an increased combustion of sugar. Its diminution, he explains, is caused by a re-formation of sugar from fat which takes place at the same time and is accompanied by an increased consumption of oxygen.

In *sleep* metabolism decreases as compared with that during waking hours, and the most essential reason for this is the muscular inactivity during sleep. The investigations of RUBNER upon a dog, and of JOHANS-

[1] See footnote 2, page 868. [2] Pflüger's Arch., 49. [3] Arch. de Physiol. (5), 8, 572.

SON [1] upon human beings, teach us that if the muscular work is eliminated the metabolism during waking hours is not greater than in sleep.

The action of *light* also stands in close connection with the question of the action of muscular work. It seems positively proven that metabolism is increased under the influence of light. Most investigators, such as SPECK, LOEB, and EWALD,[2] consider that this increase is due to the movements caused by the light or an increased muscle tonus, and in man an increase in metabolism under the influence of light with complete rest has not been observed. Divergent results have been obtained in animals, and our knowledge of the truth is not yet complete.[3]

Mental activity does not seem to have any influence on metabolism according to the means at hand for studying this influence.

The Action of the External Temperature also stands in close relation to muscular work, namely to the question as to whether the chemical heat regulation is independent of the muscular activity. In this case we must differentiate between cold-blooded and warm-blooded animals. In the first the metabolism rises with an increase in the surrounding temperature, while in the second group the conditions are different. The experiments of SPECK, LOEWY and JOHANSSON[4] on human beings have shown that the lowering of the external temperature is without influence upon the extent of metabolism (measured by the gas exchange) only as long as all natural and non-voluntary movements of the muscles are excluded. A chemical heat regulation, i.e., a rise in metabolism without noticable movements of the muscles, is not accepted in man, or at least it has not been proven. The heat regulation, in man, at lower temperatures seems to be brought about by the natural or reflex production of muscle action, nor has a chemical heat regulation in the reverse sense, namely, a fall in the catabolism by raising the external temperature, been shown in man. On the contrary in several cases a small rise in the metabolism has been observed on raising the temperature above the zone within which the fundamental catabolism of the individual showed its minimum (VOIT, RUBNER [5]). The investigations of EYKMAN [6] upon inhabitants of the tropics also show the same result, namely, that in human beings no appreciable chemical heat regulation occurs.

In animals, a rise in the external temperature causes to a certain extent

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[1] Rubner, Ludwig-Festschr., 1887; Loewy, Berl. klin. Wochenschr., 1891, 434; Johansson, Skand. Arch. f. Physiol., 8.

[2] Speck, l. c.; Loeb, Pflüger's Arch., **42**; Ewald, Journ. of Physiol., **13**.

[3] See larger handbooks for the literature on this question.

[4] Speck, l. c.; Loewy, Pflüger's Arch., **46**; Johansson, Skand. Arch. f. Physiol., **7**.

[5] C. Voit, Zeitschr. f. Biol., **14**; Rubner, Arch. f. Hyg., **38**.

[6] Virchow's Arch., **133**, and Pflüger's Arch., **64**.

a rise in the metabolism; the conditions on lowering the external temperature are rather more complicated, and a chemical heat regulation in the strict sense is difficult of exclusion, especially in small animals. If the natural muscular activity is eliminated by poisoning with curare or by section of the spinal cord, then, as shown by PFLÜGER[1] and his pupils, the warm-blooded animal behaves like a cold-blooded animal, and the metabolism decreases parallel with the body temperature. In normal animals, on the contrary, the body temperature can be kept constant, on lowering the external temperature, by an increased metabolism. On sufficiently cooling the body the temperature sinks irrespective of the increased metabolism, and at a certain limit of temperature the catabolism of substance is still lower with decreasing temperature.

A very interesting and important question is the action of high altitude upon the oxidation processes, the economy of temperature, the protein exchange and the general metabolism. The results of the laborious and important investigations on this subject may be found in the large work of N. ZUNTZ, A. LOEWY, F. MÜLLER and W. CASPARI.[2]

That the *ingestion of food* raises the metabolism has been known for a rather long time, and this has been studied by ZUNTZ, v. MERING, MAGNUS-LEVY, VOIT, RUBNER, JOHANSSON and collaborators and also by HEILNER.[3] It follows from these investigations that this rise in metabolism, which in man, on sufficient supply of food, amounts to a rise of 10–15 per cent of the basal requirement and with abundant supply of food may be still larger (35 per cent in the researches of JOHANSSON, TIGERSTEDT and collaborators), has a double cause, namely, partly a digestion work (ZUNTZ) and partly a chemical decomposition (specific dynamic action of RUBNER) which takes place at the same time.

The sum of all the work which is necessary for the chemical transformation of the foods, as well as for the mechanical division and transportation of the food in the intestinal canal, is called the *digestion work* by ZUNTZ. That such work exists has been shown by ZUNTZ and v. MERING by comparative tests of the different action upon metabolism by foods introduced per os and intravenously, and recently COHNHEIM[4] has shown that in sham feeding an increased catabolism of non-nitrogenous body

[1] See footnote 2, page 562.

[2] Hohenklima und Bergwanderungen in ihrer Wirkung auf den Menschen, Berlin, 1906.

[3] Zuntz and v. Mering, Pflüger's Arch., **15**: Zuntz, Naturw. Rundschau, **21** (1906), with Hagemann, l. c., with Magnus-Levy, Pflüger's Arch., **49**; Magnus-Levy, *ibid.*, **55**, and v. Noorden's Handbuch; Voit, Hermann's Handbuch, **6**; Rubner, Zeitschr. f. Biol., **19** and **21**, and Arch. f. Hyg., **66**; Johansson, Skand. Arch. f. Physiol., **21**, with Koraen, *ibid.*, **13**; Heilner, Zeitschr. f. Biol., **48** and **50**.

[4] Arch. f. Hyg., **57**.

constituents took place. The influence of digestion work in ZUNTZ's sense is especially apparent in herbivora, in which this work, according to ZUNTZ and collaborators, may amount to the consumption of more than 50 per cent of the total energy content of the raw fodder.

On partaking of large amounts of food, especially proteins, by carnivora, the digestion work in the above sense is not sufficient to account for the increase in metabolism, and in these cases, besides this, we must accept an increase in the chemical transformation process in the animal body brought on by the foodstuffs in an unknown manner (specific dynamic action of foodstuffs, according to RUBNER). The only real difference in opinion between the various experimenters consists, so far as HAMMARSTEN can see, in that according to the ZUNTZ school, normally on supplying sufficient food it is the digestion work in the above sense, which chiefly causes the rise in metabolism after taking food, while according to the views of VOIT-RUBNER, with which HEILNER agrees, it is on the contrary the specific dynamic action.

That the proteins or their cleavage products cause a specific dynamic action seems to be generally accepted. It is difficult to decide how far the fats have such an action while, from the investigations of JOHANSSON,[1] there is no doubt that the carbohydrates have a specific dynamic action.

JOHANSSON, who has studied the rise in the CO_2 elimination after the introduction of carbohydrates, found that this rise was proportional to the sugar supply up to a maximal limit, but that this rise was considerably less, or indeed absent when the store of glycogen was diminished. This behavior, as well as the circumstance that levulose increased the CO_2 elimination nearly twice that of glucose, cannot be explained by digestion work, which indicates a specific dynamic action of carbohydrates.

The investigations of JOHANSSON, HELLGREN and GIGON[2] have also shown that the foodstuffs, at least in the first hours after the taking of food, act for each other in the metabolism, but not according to their isodynamic values, and that they probably first pass into the various depots of the body and then are transformed by the various tissues. JOHANSSON and KORÀEN have in a similar manner found that the muscles in their work do not probably take the carbohydrate directly from the intestine, but first, after their transformation into glycogen, from the supply.

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[1] Skand. Arch. f. Physiol., 21.

[2] Johansson with Hellgren, Hammarsten's Festschrift, 1906; Gigon, Skand. Arch. f. Physiol., 21.

V. THE NECESSITY OF FOOD BY MAN UNDER VARIOUS CONDITIONS.

Various attempts have been made to determine the daily quantity of organic food needed by man. Certain investigators have calculated from the total consumption of food by a large number of similarly fed individuals—soldiers, sailors, laborers, etc.—the average quantity of foodstuffs required per head. Others have calculated the daily demand for food from the quantity of carbon and nitrogen in the excreta or calculated it from the exchange of force of the persons experimented upon. Others, again, have calculated the quantity of nutritive material in a diet by which an equilibrium was maintained in the individual for one or several days between the consumption and the elimination of carbon and nitrogen. Lastly, still others have quantitatively determined during a period of several days the organic foodstuffs daily consumed by persons of various occupations who chose their own food, by which they were well nourished and rendered fully capable of work.

Among these methods a few are not quite free from objection, and others have not as yet been tried on a sufficiently large scale. Nevertheless the experiments collected thus far serve, partly because of their number and partly because the methods correct and control one another, as a good starting-point in determining the diet of various classes and similar questions.

If the quantity of foodstuffs taken daily be converted into calories produced during physiological combustion, we then obtain some idea of the sum of the chemical energy which under varying conditions is introduced into the body. It must not be forgotten that the food is never completely absorbed, and that undigested or unabsorbed residues are always expelled from the body with the feces. The gross results of calories calculated from the food taken must therefore, according to RUBNER, be diminished by at least 8 per cent. This figure is true at least when the human being partakes of a mixed diet of about 60 per cent of the proteins as animal, and about 40 per cent of the proteins as vegetable foodstuffs. With more one-sided vegetable food, especially when this is rich in undigestible cellulose, a much larger quantity must be subtracted.

The following summary contains a few examples of the quantity of food which is consumed by individuals of various classes of people under different conditions. In the last column we also find the quantity of living force which corresponds to the quantity of food in question, calculated as calories, with the above-stated correction. The calories are therefore net results, while the figures for the nutritive bodies are gross results.

	Proteins.	Fat.	Carbohydrates.	Calories.	Authority.
Soldier during peace...	119	40	529	2784	PLAYFAIR.[1]
Soldier light service....	117	35	447	2424	HILDESHEIM.
Soldier in field.....	146	46	504	2852	HILDESHEIM.
Laborer.....	130	40	550	2903	MOLESCHOTT.
Laborer at rest.....	137	72	352	2458	PETTENKOFER and VOIT.
Cabinetmaker (40 years)	131	68	494	2835	FORSTER.[2]
Young physician.....	127	89	362	2602	FORSTER.
Young physician.....	134	102	292	2476	FORSTER.
Laborer (36 years).....	133	95	422	2902	FORSTER.
English smith.....	176	71	666	3780	PLAYFAIR.
English pugilist.....	288	88	93	2189	PLAYFAIR.
Bavarian wood-chopper.	135	208	876	5589	LIEBIG.
Laborer in Silesia.....	80	16	552	2518	MEINERT.[3]
Seamstress in London..	54	29	292	1688	PLAYFAIR.
Swedish laborer.....	134	79	485	3019	HULTGREN and LANDERGREN.[4]
Japanese student.....	83	14	622	2779	EIJKMAN.[5]
Japanese shopman.....	55	6	394	1744	TAWARA.[5]

We have a very large number of complete investigations upon the diet of people of different vocations in America, but they are too extensive to enter into, hence we must refer to the original publications of ATWATER.[6]

It is evident that persons of essentially different weight of body who live under unequal external conditions must need essentially different food. It is also to be expected (and this is confirmed by the table) that not only the absolute quantity of food consumed by various persons, but also the relative proportion of the various organic nutritive substances, shows considerable variation. Results for the daily need of human beings in general cannot be given. For certain classes, such as soldiers, laborers, etc., results may be given which are valuable for the calculation of the daily rations.

Based on extensive investigations and a very wide experience, VOIT has proposed the following average quantities for the daily diet of adults:

	Proteins.	Fat.	Carbodydrates.	Calories.
For men.....	118 grams	56 grams	500 grams	2810

But it should be remarked that these data relate to a man weighing 70 to 75 kilos and who was engaged daily for ten hours in not too fatiguing labor.

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[1] In regard to the older researches cited in this table we refer the reader to Voit in Hermann's Handbuch, **6**, 519.

[2] *Ibid.*, and Zeitschr. f. Biologie, **9**.

[3] Armee- und Volksernährung, Berlin, 1880.

[4] Untersuchung über die Ernährung schwedischer Arbeiter bei frei gewählter Kost, Stockholm, 1891. Maly's Jahresber., **21**.

[5] Cited from Kellner and Mori in Zeitschr. f. Biologie, **25**.

[6] Report of the Storrs Agric. expt. Station, Conn., 1891–1895, and 1896, and U. S. Depart. of Agriculture, Bull. **53**, 1898.

The quantity of food required by a woman engaged in moderate work is about four-fifths that of a laboring man, and we may consider the following as a daily diet with moderate work:

	Proteins.	Fat.	Carbohydrates.	Calories.
For women.....	94 grams	45 grams	400 grams.	2240

The proportion of fat to carbohydrates is here as 1:8–9. Such a proportion often occurs in the food of the poorer classes who chiefly live upon the cheap and voluminous vegetable food, while this ratio in the food of wealthier persons is 1:3–4. It would be desirable if in the above rations the fat were increased at the expense of the carbohydrates, but unfortunately on account of the high price of fat such a modification cannot always be made.

In examining the above figures for the daily rations it must not be forgotten that those for the various foodstuffs are gross results. They consequently represent the quantity of these which must be taken in, and not those which are really absorbed. The figures for the calories are, on the contrary, net results.

The various foods are, as is well known, not equally digested and absorbed, and in general the vegetable foods are less completely consumed than animal foods. This is especially true of the proteins. When, therefore, VOIT, as above stated, calculates the daily quantity of proteins needed by a laborer as 118 grams, he starts with the supposition that the diet is a mixed animal and vegetable one, and also that of the above 118 grams about 105 grams are absorbed. The results obtained by PFLÜGER and his pupils BOHLAND and BLEIBTREU [1] on the extent of the metabolism of proteins in man with an optional and sufficient diet correspond well with the above figures, when the unequal weight of body of the various persons experimented upon is sufficiently considered.

As a rule, the more exclusively a vegetable food is employed, the smaller is the quantity of proteins in it. The strictly vegetable diet of certain people, as that of the Japanese and of the so-called vegetarians, is therefore a proof that, if the quantity of food be sufficient, a person may exist on considerably smaller quantities of proteins than VOIT suggests. It follows from the investigations of HIRSCHFELD, KUMAGAWA and KLEMPERER, SIVÉN, and others (see page 858) that an almost complete or indeed a complete nitrogenous equilibrium may be attained by the sufficient administration of non-nitrogenous nutritive bodies with relatively very small quantities of proteins.

If we bear in mind that the food of people of different countries varies greatly, and that the individual also takes essentially different nourishment according to the external conditions of living and the influence

[1] Bohland, Pflüger's Arch., **36**; Bleibtreu, *ibid.*, **38**.

of climate, it is not remarkable that a person accustomed to a mixed diet can exist for some time on a strictly vegetable diet deficient in proteins. No one doubts the ability of man to adapt himself to a heterogeneously composed diet when this is not too difficult of digestion and is sufficient in quantity; nor can we deny that it is possible for a man to exist for a long time with smaller amounts of protein than VOIT suggests, namely 118 grams. Thus O. NEUMANN [1] experimented on himself during 764 days in three series of experiments, and his diet consisted of 7.42 grams protein, 117 grams fat, and 213 grams carbohydrates = 2367 gross calories, with a weight of 70 kilos and with ordinary laboratory work. These figures cannot be compared with those obtained by VOIT's worker, weighing 70 kilos, whose work was harder than a tailor's and easier than a blacksmith's; for example, the work of a mason, carpenter, or cabinet-maker. The very extensive investigations recently performed by CHITTENDEN [2] on the determination of the extent of protein necessary are of great interest. These investigations, upon a total of twenty-six persons, extended over a period of five to twenty-months, and consisted of careful observations upon the manner of living, food taken, nitrogen elimination, and the ability of performing work. The individuals were divided into three groups. The first consisted of five professional men (four assistants and one professor). The second group was composed of thirteen soldiers (of the sanitary corps of the United States army) who besides their daily work were given gymnastic exercises for six months. The third group consisted of eight athletic students who were trained in different kinds of sport.

In all the persons experimented upon the original nitrogen content of the food, which corresponded to VOIT's value or were somewhat higher, was gradually reduced more or less. The total calories supplied were not increased above the original value, but rather diminished to a reasonable extent. The bodily as well as the mental ability was repeatedly tested. As it is not possible to enter into the details of the investigation the following will be sufficient to show the results. With a diet corresponding to VOIT's values the amount of urine nitrogen per day is 16 grams, corresponding to a total protein catabolism in the body of 100 grams or 1.43 grams per kilo. The corresponding results for the above three groups may be found in the following table, where for comparison HAMMARSTEN also includes the figures for VOIT's diet:

	Urine Nitrogen.		Catabolized Protein.		Protein per Kilo.	
	Min.	Max.	Min.	Max.	Min.	Max.
Group 1	5.69	8.99	35.6	56.19	0.61	0.86
Group 2	7.03	8.39	43.9	52.44	0.74	0.87
Group 3	7.47	11.06	46.7	69.10	0.75	0.92
Voit's figures	16		100		1.43	

[1] Arch. f. Hygiene, 45.

[2] R. H. Chittenden, Physiological Economy in Nutrtition, New York, 1904.

The chief results from these investigations are that on partaking of amounts of protein much smaller than VOIT's figures, without changing the original supply of calories and indeed diminishing the same, the persons experimented upon remained not only in nitrogenous equilibrium, but remained in perfect health and were not only able to perform the ordinary work, but were indeed regularly able to perform much greater work.

From these investigations, which extended over a long period and were carried on with special care in exactitude, it cannot be denied that man can for a long time exist with much smaller quantities of protein than VOIT's figures call for, which is also derived from the experience of vegetarians and from people living almost entirely upon vegetable food. On the other hand it must not be forgotten that VOIT's figures represent average results not theoretically necessary, but which have been shown to be the actual diet developed from habit, custom, conditions of life and climate, with sufficient nourishment and free selection for centuries in Middle and North Europe. A rational change in this food requirement based upon scientific facts is just as difficult to determine as it is to carry out practically. Certain standard figures for the general needs of nutrition cannot be established because the conditions in various countries are different and must necessarily be so. The numerous compilations (of ATWATER and others [1]) on the diet of different families in America have given the figures 97–113 grams protein for a man, and the very careful investigations of HULTGREN and LANDERGREN have also shown that the laborer in Sweden with moderate work and an average body weight of 70.3 kilos, with optional diet, partakes 134 grams protein, 79 grams fat, and 522 grams carbohydrates. The quantity of protein is here greater than is necessary, according to VOIT. On the other hand LAPICQUE [2] found 67 grams protein for Abyssinians and 81 grams for Malaysians (per body weight of 70 kilos), materially lower figures.

If we compare the figures on page 874 with the average figures proposed by VOIT for the daily diet of a laborer, it would seem at the first glance as if the food consumed in certain cases was considerably in excess of the need, while in other cases, as, for instance, that of a seamstress in London, it was entirely insufficient. A positive conclusion cannot, therefore, be drawn if we do not know the weight of the body, as well as the labor performed by the person, and also the conditions of living.

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[1] Atwater, Report of the Storrs Agric. Expt. Station, Conn., 1891–1895 and 1896; also Nutrition investigations at the University of Tennessee, 1896 and 1897; U. S. Dept. of Agriculture, Bull. **53**, 1898. See also Atwater and Bryant, *ibid.*, Bull. **75**; Jaffa, *ibid.*, **83**; Grindley, Sammis, and others, *ibid.*, **91**.

[2] Hultgren and Landergren, l. c.; Lapicque, Arch. de Physiol. (5), 6.

It is certainly true that the amount of nutriment required by the body is not directly proportional to the body weight, for a small body consumes relatively more substance than a larger one, and varying quantities of fat may also cause a difference; but a large body, which must maintain a greater quantity, consumes an absolutely greater amount of substance than a small one, and in estimating the nutritive need one must also always consider the weight of the body. According to VOIT, the diet for a laborer with 70 kilos body weight requires 40 calories for each kilo. EKHOLM[1] calculates, basing it upon his experiments, that for a man weighing 70 kilos, busied with reading and writing, the net calories are 2450 and the gross calories 2700, or 35 and 38.6 calories per kilo. In the ordinary sense for a resting man the general food requirement is calculated in round numbers as 30 calories for every kilo. The minimum figure for metabolism during sleep and in as complete rest as possible has been found by SONDÉN, TIGERSTEDT and JOHANSSON[2] to be 24–25 calories.

As several times stated above, the demands of the body for nourishment vary with different conditions of the body. Among these conditions two are especially important, namely, work and rest.

In a previous chapter, in which muscular labor was spoken of, it was seen that all foodstuffs have almost the same power of serving as a source of muscular work, and that the muscles, it seems, select that foodstuff which is supplied to them in the greatest quantity. As a natural sequence it is to be expected that muscular activity requires indeed an increased supply of foodstuffs, but no essential change in their relation as compared to rest.

Still this does not seem to hold true in daily experience. It is a well-known fact that hard-working individuals—men and animals—require a greater quantity of proteins in the food than less active ones. This contradiction is, however, only apparent, and it depends, as VOIT has shown, upon the fact that individuals used to violent work are more muscular. For this reason a person performing severe muscular labor requires food containing a larger proportion of proteins than an individual whose occupation demands less violent exertion. Another fact is that the diet rich in proteins is often concentrated and less bulky, and also that in many cases of training a diet containing as little fat as possible is selected.

If we compare the results for the needs of food in work and rest which are obtained under conditions which can be readily controlled, it is found that the above statements are in general confirmed. As example of this

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[1] Skand. Arch. f. Physiol., **11**.

[2] Sondén and Tigerstedt, Skand. Arch. f. Physiol., **6**; Johansson, *ibid.*, **7**; Tigerstedt, Nord. Med. Arkiv. Festband, 1897.

the following tables give the rations of soldiers in peace and in the field and the average figures from the detailed data of various countries.[1]

	A. Peace Ration.			B. War Ration.		
	Proteins.	Fat.	Carbohydrates.	Proteins.	Fat.	Carbohydrates
Minimum	108	22	504	126	38	484
Maximum	165	97	731	197	95	688
Mean	130	40	551	146	59	557

The following figures for the daily ration are obtained from the above averages:

	Proteins.	Fat.	Carbohydrates.	Calories.
In peace	130	40	551	2900
In war	146	59	557	3250

If we calculate the fat in its equivalent quantity of starch, then the relation of the proteins to the non-nitrogenous foods is:

In peace	1:4.97
In war	1:4.79

The relation in both cases is nearly the same. Similar results are obtained when we start with Voit's figures for a soldier in manœuver A (hard work) and B (strenuous work) in war.

	Proteins.	Fat.	Carbohydrates.	Calories.
A	135	80	500	3013
B	145	100	500	3218

The relation here, when the fat is recalculated as starch, in both cases is the same, or equal to 1:5

If we calculate that portion of the total calories supplied which falls to each group of the foodstuffs, it is found that 16–19 per cent comes from the protein in rest as well as with medium and strenuous work. For the fat and the carbohydrates the variations are greater; the chief quantity of calories comes from the carbohydrates. Of the total calories 16–30 per cent comes from the fat and 50–60 per cent from the carbohydrates.

The importance of the food-demand for working individuals is shown by the figures given on page 874 for a wood-chopper in Bavaria. A need of more than 4000 calories occurs but seldom, and with very hard work the demand may rise even to 7000 calories (Atwater and Bryant, Jaffa [2]).

As more work requires an increase in the absolute quantity of food, so the quantity of food must be diminished when little work is performed.

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[1] Germany, Austria, Switzerland, France, Italy, Russia, and the United States. It is not known by the author whether these figures have been changed in the last few years in the various countries, and hence whether they must be modified or not.

[2] See footnote 1, page 877.

The question as to how far this can be done is of importance in regard to the diet in prisons and poorhouses. We give below the following as example of such diets:

	Proteins.	Fat.	Carbohydrates.	Calories.	
Prisoner (not working). .	87	22	305	1667	SCHUSTER.[1]
Prisoner (not working). .	85	30	300	1709	VOIT.
Man in poorhouse.....	92	45	332	1985	FORSTER.[2]
Woman in poorhouse....	80	49	266	1725	FORSTER.

The figures given by VOIT are, he says, the lowest reported for a non-working prisoner. He considers the following as the lowest diet for old non-working people:

	Proteins.	Fat.	Carbohydrates.	Calories.
Men.....	90	40	350	2200
Women.....	80	35	300	1723

In calculating the daily diet it is in most cases sufficient to ascertain how much of the various foodstuffs must be administered to the body in order to keep it in the proper condition to perform the work required of it. In other cases it may be a question of improving the nutritive condition of the body by properly selected food; and there also are cases in which it is desired to diminish the mass or weight of the body by an insufficient nutrition. This is especially the case in obesity, and all the dietaries proposed for this purpose are chiefly starvation cures, which is readily apparent if we study such dietaries.

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[1] See Voit, Unters. der Kost, München, 1877, page 142. See also Hirschfeld, Maly's Jahresber., **30.**

[2] Voit, Unters. der Kost, page 186.

TABLE I.—FOODS.[1]

1. Animal Foodstuffs.	1000 Parts contain						Relation of 1:2:3.		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	2	3
a. MEAT WITHOUT BONES.									
Fat beef [2]	183	166		11	640		100	90	0
Beef (average fat [1])	196	98		18	688		100	50	0
Beef [2]	190	120		18	672		100	63	0
Corned beef (average fat)	218	115		117	550		100	53	0
Veal	190	80		13	717		100	42	0
Horse, salted and smoked	318	65		125	492		100	20	0
Smoked ham	255	365		100	280		100	143	0
Pork, salted and smoked [3]	100	660		40	130		100	660	0
Meat from hare	233	11		12	744		100	5	0
" " chicken	195	93		11	701		100	48	0
" " partridge	253	14		14	719		100	6	0
" " wild duck	246	31		12	711		100	13	0
b. MEAT WITH BONES.									
Fat beef [2]	156	141		9	544	150	100	90	0
Beef (average fat [1])	167	83		15	585	150	100	49	0
Beef, slightly corned	175	93		85	480	167	100	53	0
Beef, thoroughly corned	190	100		100	430	180	100	53	0
Mutton, very fat	135	332		8	437	88	100	246	0
" average fat	100	100		10	520	150	100	100	0
Pork, fresh, fat	100	400		5	365	70	100	400	0
" corned, fat	120	540		60	200	80	100	450	0
Smoked ham	200	300		70	340	90	100	150	0
c. FISHES.									
River eel, fresh, entire	89	220		6	352	333	100	246	0
Salmon, " "	121	67		10	469	333	100	56	0
Anchovy, " "	128	39		11	489	333	100	31	0
Flounder, " "	145	14		11	580	250	100	9	0
River perch, fresh, entire	100	2		8	440	450	100	2	0
Torsk, " "	86	1		8	455	450	100	1	0
Pike, " "	82	1		6	461	450	100	1	0
Herring, salted, entire	140	140		100	280	340	100	100	0
Anchovy, " "	116	43		107	334	400	100	37	0
Salmon (side), salted	200	108		132	460	100	100	54	0
Kabeljau (salted haddock)	246	4		178	472	100	100	1	0
Codfish (dried ling)	532	5		106	257	100	100	1	0
" (dried torsk)	665	10		59	116	150	100	1	0
Fish-meal from variety of GADUS	736	7		87	170		100	1	0

[1] The results in the following tables are chiefly compiled from the summary of ALMÉN and of KÖNIG. We here designate as "waste" that part of the foods which is lost in the preparation or that which is not used by the body; for instance, bones, skin, egg-shells, and the cellulose vegetable foods.

[2] Meat such as is ordinarily sold in the markets in Sweden.

[3] Pork, chiefly from the breast and belly, such as occurs in the rations of Swedish soldiers.

TABLE I.—FOODS—(*Continued*).

1. Animal Foodstuffs.	1000 Parts contain						Relation of 1:2:3.		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
d. Inner Organs (Fresh).									
Brain	116	103		11	770		100	89	0
Beef-liver	196	56	11	17	720		100	28	0
Beef-heart	184	92		10	714		100	50	0
Heart and lungs of mutton	163	106		10	721		100	65	0
Veal-kidney	221	38		13	728		100	17	0
Ox tongue (fresh)	150	170		10	670		100	113	0
Blood from various animals (average results)	182	2		9	807		100	1	0
e. Other Animal Foods.									
Variety of pork-sausage (Mettwurst)	190	150		50	610		100	79	0
Same for frying	220	160		55	565		100	73	0
Butter	7	850	7	15	119		100	12100	100
Lard	3	990			7		100	33000	0
Meat extract	304			175	217				
Cow's milk (full)	35	35	50	7	873		100	100	143
" " (skimmed)	35	7	50	7	901		100	20	143
Buttermilk	41	9	38	7	905		100	22	93
Cream	37	257	35	6	665		100	695	95
Cheese (fat)	230	270	40	60	400		100	117	17
" (poor)	334	66	50	50	500		100	19	15
Whey cheese (poor)	89	70	456	56	329		100	79	512
Hen's egg, entire	106	93	4	8	654	135	100	88	4
" " without shell	122	107	5	10	756		100	88	4
Yolk of egg	160	307		13	520		100	192	0
White of egg	103	7	7	8	875		100	7	7
2. Vegetable Foodstuffs.									
Wheat (grains)	123	17	676	18	140	26	100	14	549
Wheat-flour (fine)	110	10	740	8	120	12	100	11	654
" " (very fine)	92	11	768	3	120	6	100	12	835
Wheat-bran	150	39	439	50	130	192	100	26	292
Wheat-bread (fresh)	88	10	550	17	330	5	100	11	625
Macaroni	90	3	768	8	131		100	3	853
Rye (grains)	115	17	688	18	140	22	100	15	600
Rye-flour	115	15	720	20	110	20	100	13	626
Rye-bread (dry)	114	20	725	15	110	16	100	18	634
" " (fresh, coarse)	77	10	480	16	400	17	100	14	623
" " (fresh, fine)	80	14	514	11	370	11	100	18	634
Barley (grains)	111	21	654	26	140	48	100	19	589
Scotch barley	110	10	720	7	146	7	100	9	654
Oat (grains)	117	60	563	30	130	100	100	51	481
" (peeled)	140	60	660	20	100	20	100	43	471
Corn	101	58	656	17	140	28	100	57	662
Rice (peeled for boiling)	70	7	770	2	146	5	100	10	1100
French beans	232	21	537	36	137	37	100	9	231
Peas (yellow or green, dry)	220	15	530	25	150	60	100	7	240
Flour from peas	270	15	520	25	125	45	100	6	192

TABLE I.—FOODS—(*Continued*).

2. Vegetable Foodstuffs.	1000 Parts contain						Relation of 1:2:3		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	2	3
Potatoes	20	2	200	10	760	8	100	10	1030
Turnips	14	2	74	7	893	10	100	14	529
Carrot (yellow)	10	2	90	10	873	15	100	20	900
Cauliflower	25	4	50	8	904	9	100	16	200
Cabbage	19	2	49	12	900	18	100	11	258
Beans	27	1	66	6	888	12	100	4	244
Spinach	31	5	33	19	908	8	100	16	106
Lettuce	14	3	22	10	944	7	100	21	157
Cucumbers	10	1	23	4	956	6	100	10	230
Radishes	12	1	38	7	934	8	100	8	317
Edible mushrooms (average)	32	4	60	9	877	18	100	12	188
Same dried in the air (average)	219	25	412	61	160	123	100	12	188
Apples and pears	4		130	3	832	31	100		3250
Various berries (average)	5		90	6	849	50	100		1800
Almonds	242	537	72	29	54	66	100	222	30
Cocoa	140	480	180	50	55	95	100	343	129

TABLE II.—MALT LIQUORS.

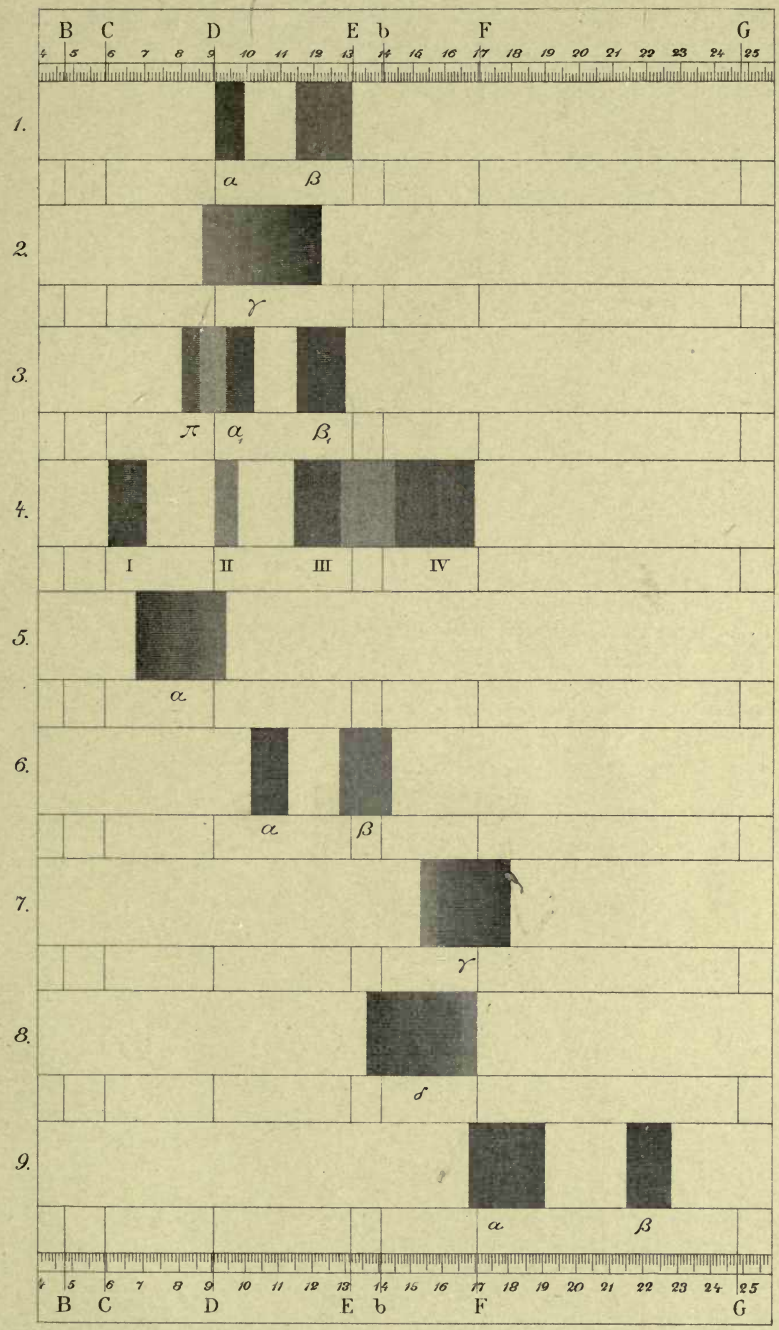
1000 Parts by Weight contain	Water.	Carbon Dioxide.	Alcohol.	Extract.	Proteids.	Sugar.	Dextrin.	Acids.	Glycerine.	Ash.
Porter	871	2	54	76	7	13		3.0	—	4
Beer (Swedish)	887		28	—	15	65		—	—	5
" (Swedish export)	885		32	—	7	73		—	—	3
Draught-beer	911	2	35	55	8	10	31	2.0	2	2
Lager-beer	903	2	40	58	4	7	47	1.5	2	2
Bock-beer	881	2	47	72	6	13	—	1.7	—	3
Weiss-beer	916	3	25	59	5	—	—	4.0	—	2
Swedish "Svagdricka"	945	—	22	—	7	23		—	—	3

TABLE III.—WINES AND OTHER ALCOHOLIC LIQUORS.

1000 Parts by Weight contain	Water.	Alcohol, Vol. Per Cent.	Extract.	Sugar.	Acid and Potassium Bitartrate.	Glycerin.	Ash	Carbon Dioxide. Vol Per Cent.
Bordeaux wine	883	94	23	6	5.9	—	2.0	
White wine (Rheingau)	863	115	23	4	5.0	—	2.0	
Champagne	776	90	134	115	6.0	1.0	1.0	60–70
Rhine wine (sparkling)	801	94	105	87	6.0	1.0	2.0	
Tokay	808	120	72	51	7.0	9.0	3.0	
Sherry	795	170	35	15	5.0	6.0	5.0	
Port wine	774	164	62	40	4.0	2.0	3.0	
Madeira	791	156	53	33	5.0	3.0	3.0	
Marsala	790	164	46	35	5.0	4.0	4.0	
Swedish punch	479	263	—	332	—	—	—	
Brandy	—	460	—	—	—	—	—	
French cognac	—	550	—	—	—	—	—	
Liqueurs	—	442–590	—	260–475	—	—	—	

SPECTRUM PLATE.

1. Absorption spectrum of a solution of *oxyhæmoglobin.*
2. Absorption spectrum of a solution of *hæmoglobin,* obtained by the action of an ammoniacal ferro-tartrate solution on an oxyhæmoglobin solution.
3. Absorption spectrum of a faintly alkaline solution of *methæmoglobin.*
4. Absorption spectrum of a solution of *hæmatin* in ether containing oxalic acid.
5. Absorption spectrum of an alkaline solution of *hæmatin.*
6. Absorption spectrum of an alkaline solution of *hæmochromogen,* obtained by the action of an ammoniacal ferro-tartrate solution on an alkaline-hæmatin solution.
7. Absorption spectrum of an acid solution of *urobilin.*
8. Absorption spectrum of an alkaline solution of *urobilin* after the addition of a zinc-chloride solution.
9. Absorption spectrum of a solution of *lutein* (ethereal extract of the egg-yolk).


B C D E b F G
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
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